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OMICS in Periodontics - A fad in the frontline

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Abstract

OMICS refers to a field of study in biology ending in omics, such as genomics, proteomics or metabolomics. The related suffix – omics used to address the objects of study of fields such as the genome, proteome or metabolome respectively. Omics aims at the collective characterization and qualification of pools of the biological molecules that translate into the structure, function and dynamics of the organisms. The current article reviews on the key concepts, history, principles and clinical application of omics in Periodontics.

Keywords: OMICS, Periodontitis, Salivary diagnostics, Biology

Introduction

Salivary diagnostics is a dynamic and emerging field in the diagnosis of oral and systemic diseases. Saliva reflects the physiologic state of the body, including emotional, endocrinal, nutritional, and metabolic variations. The collection of saliva samples is noninvasive, safe, and inexpensive. Saliva has enormous elements with diagnostic potential, omics technology made it possible to achieve the best of the saliva's diagnostic potential into the clinical practice.

Saliva contains a variety of biomolecules, including DNA, mRNA, micro RNA, proteins, metabolites and microbiota; changes in the salivary concentration of these biomolecules can be used to develop dysregulated biomarkers to help identify early oral and systemic diseases, evaluate disease prognosis and risk, and monitor the response to treatment. The term 'salivomics' was coined in 2008 to reflect knowledge about the various 'omics' constituents in saliva, including the genome, epigenome, transcriptome, proteome, metabolome and microbiome.

Studies have unveiled the potential to identify and measure panels of biomarkers in saliva for diagnosing periodontal diseases and monitoring progression and health. Periodontal tissue physiology and periodontal diseases are very complex, and have provided challenges along the way toward reaching the goal of chair-side salivary diagnostics for individualized treatment and maintaining periodontal health.

History

On 25th April 1953, Watson and Crick published “the molecular structure of DNA”. If the genomic era was said to have a precise birth date, it was on April 14, 2003. That was when Human Genome Project was launched with the participation of former U.S President Bill Clinton and former British PM Tony Blair which contained the complete sequencing of the human genome.

It was then realized that a new era in biological and medical sciences was beginning. This is often referred to as the ‘omics’-revolution.

On 1999 Evans and Relling, Pharmacogenomics and Pharmacogenetics introduced as new insights in to how human genetic variation influence individual drug absorption and utilization during therapy.

On Reznik 2008, oral fluids have become informative fluids that can be used for diagnostic purposes, drug therapy and a number of forensic applications. Mark Wilkins in 1986 has first coined the term proteomics which makes an analogy with genomics the study of genes and their functions. Park NJ et al 2006 had focused on Transcriptome studies mainly on mRNA and micro RNA, which are secreted from cells and enter the oral cavity from various sources, including salivary glands, gingival crevicular fluid and desquamated oral epithelial cells.

Metabolomics is the state of the art analytical techniques such as High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) or two-dimensional gas chromatography MS and nuclear magnetic resonance spectroscopy in conjunction with pattern recognition methods and can help in monitoring and discovering metabolic changes related to disease onset or therapeutic intervention. Barnes et al. have shown the feasibility of differentiating healthy and periodontal conditions by using three different metabolomics approaches. Microbiomics and metagenomics are two fields of research that have emerged to identify the presence of specific microbes in the body and understand the nature of the microbiome activity during both health and disease.

Ai J, Smith B,Wong DT,J AM Dent Assoc 2008 -2012, Coined the term ‘salivomics’ to reflect knowledge about the various ‘omics’ constituents in saliva, including the genome, epigenome, transcriptome, proteome, metabolome and microbiome. In 1997, the first nutrigenomics company was launched. In 1999, Nancy Fogg-Johnson and Alex Merolli changed the name nutrition genomic to genomics which provides powerful means of discovering hereditary factors in disease.

One of the main applications in nutrigenomics research relates to health and prevention of chronic diseases (such as e.g., cardiovascular diseases, periodontitis, metabolic syndromes, cancer, etc.) through diet. Omics technology can be applied not only for the greater understanding of normal physiological processes but also in disease processes where they play a role in screening, diagnosis and prognosis las well as aiding our understanding of the etiology of diseases. Omics strategies lend themselves to biomarker discovery as they investigate multiple molecules simultaneously.

Genomics: Large scale, high-throughput molecular analyses of multiple genes, gene products, or regions of genetic material.

Structural Genomics: The study of the physical aspects of the genome through the construction and comparison of gene maps and sequences, as well as gene discovery, localization, and characterization.

Functional Genomics: The study of the biological function of the genome by understanding what genes do and how they are regulated; includes expression profiling, the expression values for a single gene across many experimental conditions, or for many genes under a single

condition, and how such expression relates to organ dysfunction.

Clinical Genomics: The application of genomics technologies in clinical settings, such as clinical trials or primary care of patients.

Chemical Genomics (Or) Chemogenomics: The process of screening chemical compounds against genes or gene products, such as proteins or other targets. Functional analysis is used to evaluate gene response, investigate drug candidates, and identify and validate therapeutic targets. Genomics-based techniques currently employed include nucleotide polymorphisms, subtractive hybridization, microsatellite instability, DNA methylation patterns, SAGE, and microarrays.

Metabolomics: The study of the metabolite profiles in biological samples. The general aim of metabolomics is to identify, measure and interpret the complex time-related concentration, activity and flux of endogenous metabolites in cells, tissues, and other bio samples such as blood, urine, and saliva; here metabolites include small molecules that are the products of intermediary metabolism, including carbohydrates, peptides, and lipids.

Metabolome: The quantitative complement of all the low molecular weight molecules present in cells in a particular physiological or developmental state.

Lipidomics: The systems-level scale analysis of lipids and their interacting molecules.

Glycomics: The systems-level scale analysis of glycans and their interacting molecules.

Metabonomics: The quantitative measurement of the dynamic multi parametric metabolic response of living systems to pathophysiological stimuli or genetic modification (metabolic fingerprinting). Typically, involves the application of spectroscopy to study multi component measurement of bio fluids, cells, and tissues

Pharmacogenetics: The study of the impact of genetic factors on the inter individual variation in responses to drugs and drug toxicity. It describes the effects of genetic variation on pharmacokinetics and therapeutic index and includes the study of drug metabolism enzymes and drug transporters. Also referred to as gene identification for facilitating the choice of the right medicine for the right patient.

Pharmacogenomics: The study of genetic variations and their relations to drug effects and response. It describes genetic variation on pharmacodynamic variables, such as a drug's target and constituents of the target pathways.

It includes the application of tools including, but not limited to, the functional genomics toolbox of differential gene expression, proteomics, tissue immunopathology, and histopathology.

Proteomics: The large-scale, high-throughput analysis of proteins that begins with the systematic separation and identification of all proteins within a cell, tissue, or other biological sample. It involves a comprehensive study of quantitative data on the proteins of an organism under a variety of conditions (including Post synthetic modifications and interactions with other molecules).[1]

The Genetic background of an individual may influence the susceptibility to several diseases and conditions. Periodontal diseases considered to be a complex disease, caused by an overgrowth of specific Gram negative species resulting in progressive destruction of the periodontal ligament, alveolar bone with pocket formation, recession or both. It has been demonstrated that genetic variations accounts for approximately half of the variance in periodontitis. Like many other common human diseases, periodontitis appears to be influenced by more than one gene; and identification of such genes would provide a valuable tool for risk assessment.[2]

Proteins are the working parts of human cells. Almost every organic molecule in the body is either a protein or the result of a protein's activity. The word 'proteome' is a blend of the words protein and genome.[3]

The proteome is the entire complement of proteins including the modifications made to a particular set of proteins, produced by an organism or system. This will vary with time and distinct requirements, or stresses that a cell or organisms undergo.

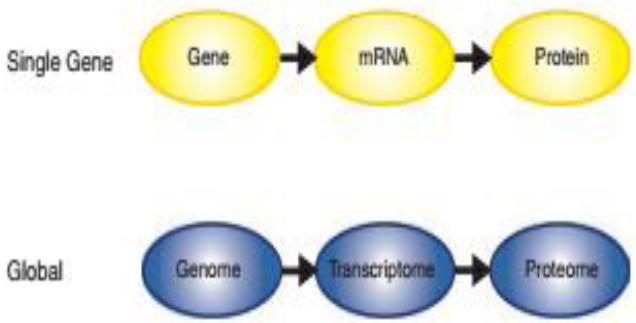

Fig. 1. Simplified flow diagram showing the contrast between traditional, gene-to-protein analysis that has been used before global analysis of gene and protein expression became widespread. The global genomic and proteomic analysis approach considers that the phenotype is attributable to not just the expressed set of proteins but also their interactions, an important analytical strategy of proteomics.

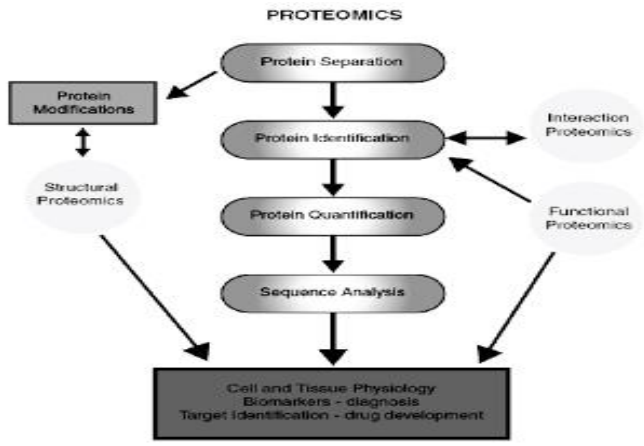

Figure 2: Protomics

Types 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 3 dimensional structures of the proteins. Structural genomics attempts to map the total repertoire of protein folds in the hope of providing 3 dimensional images for all proteins in an organism and to infer protein functions.

Interaction & functional proteomics: The functions of biological system are dependent on interactions between their components. These interactions are ultimately determined by genetic elements and selection processes. The regulation of cell metabolism involves protein interaction domains which regulates the association of polypeptides with each other and with phospholipids, small molecules or nucleic acids.[4]

Proteomics in dentistry: The two primary areas which dental proteomics have really shown as inroad are salivary diagnostics. i.e, oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel.[5]

Human saliva contains proteins that can be informative for disease detection and surveillance of oral health. Specific salivary proteomic biomarkers have been identified for 3 key features, namely the pathogenic process inflammation, collagen degradation and bone turnover. The salivary proteomic biomarkers includes anti trypsin, apolipoprotein A-I, cystatin A,SA,SA-III and SN,enolase I, hemoglobin chain, thioredoxin perioxiredoxin B, as well as prolactin-inducible protein. The proteomic approach identifies candidates from human whole saliva that may prove to be of diagnostic and therapeutic significance.[6]

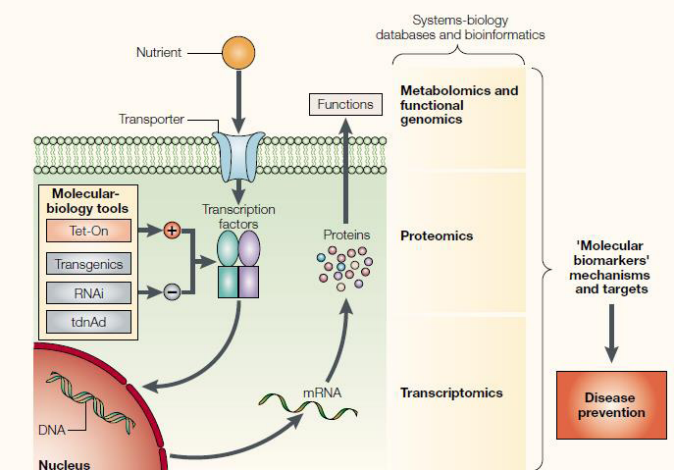

Figure 3: Proteomics in dentistry

Proteomics in periodontal challenges: Periodontal ligament fibroblast 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.[7]

Analyzing the protein expression of entire genome rather than one protein at a time was proposed by Mark Wilkins in 1994. But the complexity of the proteome seems to be the major obstacle in achieving Wilkin's idea. In addition, there is exceedingly high abundance of proteins in a cell & its function may depend not only on its abundance, post translational changes but also its cell location & association with other proteins, and all these may change in a fractional second. Further for the isolation of proteins, researchers are entirely dependent on the samples due to the unavailability of techniques like polymerase chain reaction as they have for the amplification of nucleic acid sequences.[8]

Proteomics in modern periodontics: Proteomics offers a new ray of light not only in understanding the complex interactions that the periodontal pathogens undergoes with the host both in the health and disease but also the role of periodontal ligament fibroblast & various disease related protein markers that offers an insight of the physiology of periodontal ligament.[9]

Role in periodontal pathogens: The oral ecology is a wide array of diversified communities of microorganisms that undergoes complex interactions with the host in both the healthy and diseases state. Proteomics offers an entirely new way for understanding the changes occurring as the oral microorganisms adapt to changes in the environment within their habitat in the mouth.[10]

Role in periodontal ligament (PDL) fibroblasts: To understand PDL physiology and disease related protein markers, the analysis of the entire complement of PDL fibroblast proteome is of utmost importance. Although immunological methods have been tried to study the protein expression of PDL fibroblast, this is limited to only previously identified proteins for which antibodies are available. In total 117 proteins have been identified from PDL fibroblasts which can act in reference mapping for further clinical & basic research work.[11]

Proteomic analysis of periodontogenic microorganisms: Proteomics of Porphyromonas gingivalis within a model of oral microbial Community: Oral microbial communities are considered to involve a complex dynamics in biofilms formation that develop through distinct microbial colonization.[12] With the extension of biofilm subgingivally, there is colonization of more pathogenic anaerobic gram negative microorganisms including P. gingivalis. The most remarkable property of P. gingivalis is that its pathogenecity is expressed only in mixed microbial communities. Subsratum and metabolic support is provided by the pioneering microorganisms which ultimately help in the establishment of succeeding organisms. A complex consortium is thus formed through interspecies communication and signaling molecules like short range soluble mediators, /or nutritional stimuli P.

gingivalis can accumulate into both single species and mixed species consortia with streptococcus gordonii and other related oral streptococci. This can be very well appreciated by the exclusive localization of P. gingivalis in areas of streptococcus rich plaque. In the supragingival plaque due to the aerated environment, development of more complex multispecies communities occur with predominance of Fusobacterium nucleatum, due to its oxygen scavenging property. F. nucleatum is also able to coaggregate with P.gingivalis and with oral streptococci.[13]

Biomarkers for periodontal diseases by proteomic approaches

An understanding of the human salivary proteome is a prerequisite to gain insight into the physiological and pathological processes relevant to oral health, and is crucial for the identification of meaningful biomarkers for oral diseases.[14] Periodontopathic bacteria produce virulence factors that cause degradation of host tissue either directly or activate a host response resulting in the release of biological mediators from host cells. These mediators in case of exaggerated response lead to host tissue destruction.[15] Host & bacteria derived enzymes, proteins and other inflammatory mediators appear to be hopeful salivary diagnostic biomarkers for periodontal diseases. Bacterial lipopolysaccharide & other microbial components (like bacterial DNA) trigger the innate host defense resulting in recruitment of neutrophils, monocytes & activated macrophages at the site. These host cells in turn release numerous cytokines such as prostaglandins (PGE), Tumor Necrosis Factor (TNF), interleukins IL1& IL6 which direct the inflammatory process further.[16] As a result, collagen destroying enzymes called matrix metallo proteinases carboxy terminal telopeptide and osteocalcin are then released into the surrounding area & transported through gingival crevicular fluid into the periodontal pocket. Numerous mediators of diagnostic value involved in this disease process have been shown to be correlated with the disease onset & activity. Among these mediators, host derived MMP's are considered to be key initiators of the extracellular matrix degradation associated with periodontal diseases. Both MMP1 (Interstitial collagenase) & MMP8 (Polymorphonuclear leukocyte derived collagenase) appear to be associated in periodontitis. Polymorphonuclear leukocyte derived MMP8 has been identified as a major tissue destructive enzyme during active stages of periodontitis. Therefore quantification of level of MMP8in saliva is a promising candidate for diagnosing and predicting the progression of this episodic disease. Likewise significant increase in levels of salivary IL1β & TNFα level were observed in patients with active periodontal disease sites, as these cytokines mediate osteoclastogenesis and bone breakdown.[17]

The Future of Proteomics

Customized Drugs: One of the most promising developments to come from the study of human genes and proteins has been the identification of potential new drugs for the treatment of disease. This relies on genome and proteome information to identify proteins associated with a disease, which computer software can then use as targets for new drugs. For example, if a certain protein is implicated in a disease, its 3D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. This is the basis of new drug-discovery tools, which aim to find new drugs to inactivate proteins involved in disease.

Target Gene Mechanism versus Signature Pro- file Biomarkers: It is well established that certain nutrients have direct effects on gene expression through both epigenetic mechanisms and modification of transcription factors.[18] Polyunsaturated Fatty Acids (PUFAs) are one

such example of nutrients that directly alter transcription factors through the nuclear Peroxisome Proliferator Activated Receptors (PPARs). These receptors bind to fatty acid ligands and then form a heterodimer complex with another nuclear receptor, retinoid-X- receptor. This heterodimer complex binds to specific DNA sequences to regulate gene expression. PPAR activation has been shown to modulate inflammation, including the inhibition of secretion of IL-1, 6, TNF-α by stimulated monocytes.[19]

Other nutrients alter the oxidation–reduction status of the cell to indirectly influence transcription factor activity. Many antioxidants will alter the activation status of the transcription factor nuclear factor κB, which is a key regulator of many genes.

Nutritional compounds such as n-3 fatty acids and isoflavones have been shown to alter genes that code for cytokines, growth factors, cholesterol-metabolizing enzymes and lipoproteins. There is a strong interaction between the dietary intake of PUFAs and the 5-LOX polymorphism.

The Sweet Spot for Genomic Health

"There is increasing evidence that genome instability, in the absence of overt exposure to genotoxicants, is itself a sensitive marker of nutritional deficiency," says Fenech. Fenech originated the concept of "genome health nutrigenomics," the science of how nutritional deficiency or excess can cause genome mutations at the base sequence or chromosomal level. This is critically important because increased damage to the genome is among the fundamental causes of infertility, developmental defects, cancer, and neuro degenerative diseases. Folate is among the nutrients most often cited as critical to genomic stability. The control of food intake is profoundly influenced by gene variants encoding taste receptors or those encoding a number of peripheral signaling peptides such as insulin, leptin, ghrelin, cholecystokinin, and corresponding receptors. Total dietary intake, and the satiety value of various foods, will profoundly modify the impact of these genes.

Nutrigenetics and Nutrigenomics

Nutrigenetics and nutrigenomics are defined as the science of the effect of genetic variation on dietary response and the role of nutrients and bioactive food compounds in gene expression, respectively.[20] Exploitation of this genomic information along with high-throughout 'omic' technologies allows the acquisition of new knowledge aimed at obtaining a better understanding of nutrient-gene interactions depending on the genotype with the ultimate goal of developing personalised nutrition strategies for optimal health and disease prevention.[21] There are three central factors that underpin nutrigenetics and nutrigenomics as an important science. First there is great diversity in the inherited genome between ethnic groups and individuals which affects nutrient bioavailability and metabolism. Second, people differ greatly in their food/nutrient availability and choices depending on cultural, economical, geographical and taste perception differences. Third malnutrition (deficiency or excess) itself can affect gene expression and genome stability; the latter leading to mutations at the gene sequence or chromosomal level which may cause abnormal gene dosage and gene expression leading to adverse phenotypes during the various life stages.

Nutrition and Metabolomics: Metabolomics is the latest of the 'omics' technologies and aims at identifying novel metabolic biomarkers from cells, tissues or body fluids.

It employs state of the art analytical techniques such as High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) or two-dimensional gas chromatography and nuclear magnetic resonance spectroscopy in conjunction with pattern recognition methods and can help in monitoring and discovering

metabolic changes related to disease onset or therapeutic intervention. In addition to saliva, gingival crevicular fluid is also being explored for the diagnosis of periodontal diseases. The average salivation range of an individual ranges from 0.3 to 0.7 mL/min corresponding to 1–1.5 L daily.[22]

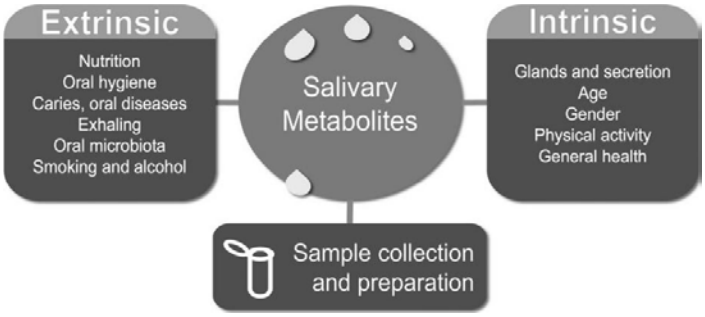

Figure 4: Nutrition and Metabolomics

Microbiomics

The Human Microbiome Project (HMP) takes a leading role in human microbiomics. It explores the role of the human microbiome in physiology, health, and disease through metagenomics research, which analyzes the genomes of specific microorganisms.[23] However, the most complicated issue that arises in microbiomics and metagenomics, is the significance of studying microbes in isolation and outside the context of their natural habitat within the human body.[24]

The analysis of the microbiome and its genomes will pave the way for more effective therapeutic and diagnostic techniques and, ultimately, contribute to the development of personalized medicine and personalized dental medicine.[25]

Transcriptomics

The transcription of specific mRNA and microRNA is altered in disease states. This is typically studied in cell populations and thus in periodontal investigations either uses biopsies of relevant oral tissues or peripheral blood leukocytes rather than oral fluids such as gingival crevicular fluid and saliva, which can be studied using proteomic and metabolomics platforms. Two major advantages that this technique provides:

(i) The ability to amplify the expressed gene products; and

(ii) The stability and uniformity of the platforms employed in identification of interesting and/or novel chemical species.

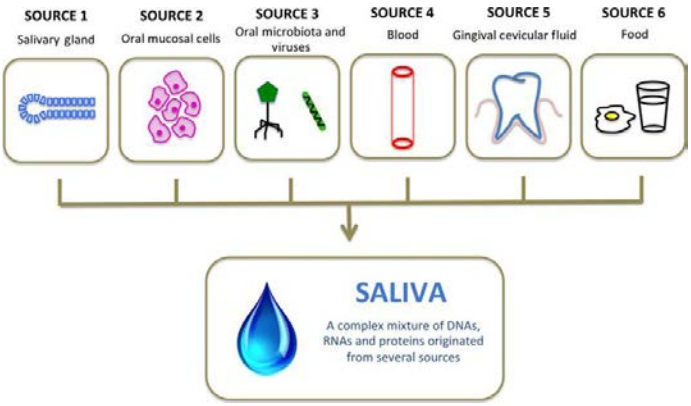

Figure 5: Transcriptomics

Salivomics

Saliva is a biofluid comprising secretions of the salivary glands (the parotid, submandibular, sublingual and other minor salivary glands), oral mucosa cells, blood and gingival crevicular fluid.

Saliva contains a variety of biomolecules, including DNA, mRNA, microRNA, proteins, metabolites and microbiota; changes in the salivary concentration of these biomolecules can be used to develop dysregulated biomarkers to help identify early oral and systemic diseases, evaluate disease prognosis and risk, and monitor the response to treatment.[26]

Salivary Transcriptome

The human salivary transcriptome was first discovered in our laboratory using microarray technology, allowing high-throughput analysis.[27] Transcriptome studies have focused mainly on mRNA and microRNA, which are secreted from cells and enter the oral cavity from various sources, including salivary glands, gingival crevicular fluid and desquamated oral epithelial cells. The

transcription of specific mRNA and microRNA is altered in disease states.[28]

Pharmacogenomics

Pharmacogenetics is an old discipline. One many distinguish **pharmacogenetics** (the study of a single gene) and **pharmacogenomics** (study of many genes or entire genomes) or use pharma genomics for approaches that go beyond DNA to include mRNA and proteins. Today, it is possible to assess entire pathways that might be relevant to disease or to drug response at the DNA, mRNA and protein levels. Eventually, the entire genome, transcriptome and proteome will be available. Therefore, pharmacogenetics/genomics and disease genetics/genomic are undergoing similar transitions, with a shift in focus from Mendelian examples to more complex modes of genetic causation.

Discussion

The etiologic basis for most human diseases can be defined in terms of gene-gene and/gene environment interactions have fundamentally altered our perception of human diseases. It was the practical ability to apply genetic information to disease paradigms that was transforming their approach to the diagnosis and treatment of human disease. Currently, an unprecedented combination of academic, industrial, financial, and governmental resources were been focused on the study of the genetic basis for human diseases. As a result, the field of genetics had been transformed the academic pursuit to a vigorous and applied science in which the issues and principles of medical genetics were coming to bear across all health care disciplines. Omics analyses, including the systematic cataloging of messenger RNA and microRNA sequences or DNA methylation patterns in a cell population, organ, or tissue sample, allow for an unbiased, comprehensive genome-level analysis of complex diseases, offering a large advantage over earlier "candidate" gene or pathway analyses. A major issue when inferring biological information from high-throughput –omics studies were the fact that the sheer volume of high-dimensional data generated by contemporary technology is not appropriately analyzed using common statistical methods employed in the biomedical sciences.

The highly diverse oral microbiome represents one of the most studied human microbiomes and was now accepted to have important roles in host health and disease.

Periodontitis is a chronic inflammatory disease, and its persistent nature could also exert a significant systemic impact on health, by serving a risk factor for atherosclerosis, chronic obstructive pulmonary disease, diabetes, adverse pregnancy outcomes, and rheumatoid arthritis. The traditional method for periodontal disease management involves techniques targeting the bacteria/pathogens. These have limitations, such as recurrence of the disease and bacterial resistance. Thus, developing new therapeutic strategies for chronic inflammation based on regulation of the host innate immune response is highly desirable. Knowledge about alterations in histone modifications, DNA methylation, and microRNA regulation will provide a better understanding of the molecular basis for various chronic inflammatory diseases. Progress in studies of epigenetic alterations during the inflammatory response opens opportunities for the development of effective medications for specific targets. The use of proteomics and gene expression will advance the diagnosis and treatment of periodontal diseases. However, its application into the field of dentistry depends on how best oral health care practitioners will incorporate this into their practice as it requires a thorough knowledge of human genetics and application of new diagnostic and therapeutic technologies. The current literature on the relationship

between diet and periodontal disease is largely inconclusive; this is most likely due to a lack of clarity in assessment of nutritional status. To date, there is no single biomarker that is specific for periodontal disease. Therefore, there is strong potential for the use of microbial and host response biomarkers in combination to enhance identification of the disease process, given the multifactorial nature of periodontal disease. It is envisioned that proteomic salivary tools can be used to identify markers for early detection, disease progression and therapy monitoring of patients with periodontal disease. While many questions remain, the potential advantages of salivary analysis for the diagnosis of periodontal diseases suggest that further studies are warranted. Integrating these new salivary diagnostic methods into clinical practice is important to aid dental professionals in making essential health related decisions for patients.

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