Review Article

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SALIVARY PROTEOMICS: A PROMISING TECHNOLOGY IN ORAL ONCOLOGY:A BRIEF REVIEW

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ABSTRACT:

Early stage detection of cancer is the key to provide a better outcome for therapeutic intervention. Most routine screening and diagnosis tools for oral cancer lack sufficient sensitivity and specificity and sometimes they are invasive. Proteomic technologies hold recently great promise in the search of new clinical biomarkers for the early detection and diagnosis of cancer. Proteomic study of human body fluid (saliva) holds promise as a non-invasive method to identify biomarkers for human oral cancer. This review article provides a brief overview of the importance of salivary proteome in early detection of oral oncology.

Keywords: proteomics, oral cancer, protein, saliva, biomarkers.

INTRODUCTION:

Improvements in technology that allow miniaturization and high-throughput analyses of thousand of genes and gene products have changed the focus and scope of research and development in both academic and industry. It is now possible to study entire proteomes with the goals of elucidating protein expression, sub cellular localization, biochemical activities and their regulation. Alterations in different cell types in disease states can be revealed.[1]

Proteomics is the study of the proteome, the protein complement of the genome[2].This field of research has acquired the name 'Proteomics". 'PROTEins expressed by the genOME' have been termed 'Proteome', a term firstly used in the public by Mare Wilkins at the first Siena proteomic conference in 1994.It is a field that Anderson and Anderson have defined as: 'the use of quantitative protein-level measurement of gene expression to characterize biological process and

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decipher the mechanisms of gene expression control'.[2]

Proteomics is broadly defined as a collection of scientific approaches and technologies to characterize the protein content and its modification within cells, tissues, body fluids and whole organism at a particular stage or condition.[3]It is a foremost approach for comprehensive study of the structure and function of all proteins expressed in a biological system. Proteomic is a powerful approach to discover protein biomarkers in human diseases and cancer for clinical and diagnostic applications.[4]

Oral Cancer is a major global public health problem with 300,000 new cases diagnosed annually. It is the most common cancer in males and third commonest cancer in females in India. Despite rapid advances in multimodality therapy, the morbidity and mortality rates of this devastating disease have not improved in decades. Early detection of oral cancer is the most effective way to improve survival. The treatment planning of oral cancer is mainly based on the tumor, node and metastasis (TNM) classification and histopathological diagnosis. These methods are subjective and often lack sensitivity to detect the disease in early stages. Furthermore, these methods do not reflect the aggressiveness of tumors, prognosis and response to therapy. Therefore, there is an urgent need to develop biomarkers to: identify high risk individuals, improve cancer detection in early stages, predict disease outcome and response to therapy. Rapid advances in high throughput proteomic technologies have paved the way for better understanding the molecular pathogenesis of oral cancer and identify candidate biomarkers for oral cancer.[5]It has been widely accepted that a major challenge to cancer proteomics is the integration of biochemical, genetics and proteomics data in the detection of biomarkers to provide impetus for the next level of clinical application.

Importance of Saliva to be used as a biomarker

Whole human saliva possesses tremendous potential in clinical diagnostics particularly for conditions within the oral cavity such as oral cancer.[6]Non-invasive, inexpensive and accurate tests, ones which foster early and frequent screening, would, if available, transform the early detection of oral cancer. Whole saliva collection is non invasive, providing inexpensive collection of ample amounts of sample for analysis in an on-demand manner. Protein biomarkers in whole saliva could fulfil this need if their abundance levels distinguish patients with pre-malignant oral lesions from those with malignant lesions.[7]

With expanded research and improved proteomic technologies for measuring salivary biomarkers at the molecular level, the field of salivary diagnostics holds tremendous promise for the long- term goal of developing clinically validated,saliva-based tests for health surveillance and early detection of oral diseases.This highly successful effort led to the identification of 1166 proteins in human saliva,a landmark accomplishment that has started to provide definition and boundaries for clinical diagnostic applications.This toolbox is

known as the salivary "proteome," a complete set of proteins expressed and modified following their expression by the genome.This salivary proteome is used successfully to identify highly discriminatory biomarkers for the detection of oral cancer.[8]

PROTEOMIC WORKING PRINCIPLE

The proteome represents the complete set of proteins encoded by genome and proteomics is the study of the proteome that investigates the cellular levels of all the isoforms and post-translational modifications of proteins that are encoded by the genome of the cell under a given set of circumstances. Whilst a genome is more or less static, the protein levels in a cell can change dramatically as genes get turned on and off during the cells response to its environment. The proteomic analysis can ascertain function by either looking for changes in the expression of either all or subset of proteins, or by identifying binding partners for particular proteins and seeing how their interaction is affected by biological perturbation.

Proteomic analysis involves the following stages :

i**. Separation of proteins**: Before analyzing protein expression and abundance levels, proteins have to be isolated into a purified state. This is generally done by using two dimensional polyacrylamide gel electrophoresis (2D-PAGE). To resolve the complex composition of saliva, 2D- PAGE, allows separation not only of different molecules of similar molecular weights, but also of different modification patterns or isoforms of the same protein.

ii**. Analysis of comparative expression**: Once separated, it is necessary to carry out some form of analysis to assess the relative abundance of the protein present.

iii. **Identification of protein species**: Once a set of proteins showing differences in abundance between two or more states has been identified, mass spectrometric analysis is to be used to determine their identities. Proteins that are primarily identified by mass spectrometry (MS) can be further characterized by ionization methods such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI)

In 2D-PAGE, the proteins are initially separated by isoelectric focusing in the first dimension according to charge and then by SDS-PAGE in the second dimension according to size. This type of separation has the capacity to resolve complex protein mixtures, thus permitting analysis of hundreds of proteins at a time. For visualization, staining methods like Coomassie staining, silver staining can be used.

Mass spectrometry is a powerful technique used for the identification of proteins from two dimensional cells. The ionization methods such as MALDI (matrix assisted laser desorption ionization) and ESI (electrospray ionization) were developed by Karas & Hillenkomp (1988) and Fenn et al (1989) respectively. The combination of either of the above mass spectrometric techniques with the separation of proteins

by 2D-PAGE is an established method of proteome analysis. In both the cases identification takes place at the peptide level and is therefore necessary to convert proteins in the excised gel pieces into peptides which can be extracted for analysis. Peptides can also be analyzed by MALDI to produce peptide mass fingerprints which are then matched against protein databases in order to identify corresponding proteins. It is also useful to identify which peptides in a tryptic digest have undergone post-translational modification.[9]

Mass Spectrometry-based proteomics can be used for early discovery steps of biomarkers and their validation but also for clinical diagnosis and prognosis as an endpoint clinical assay [10].

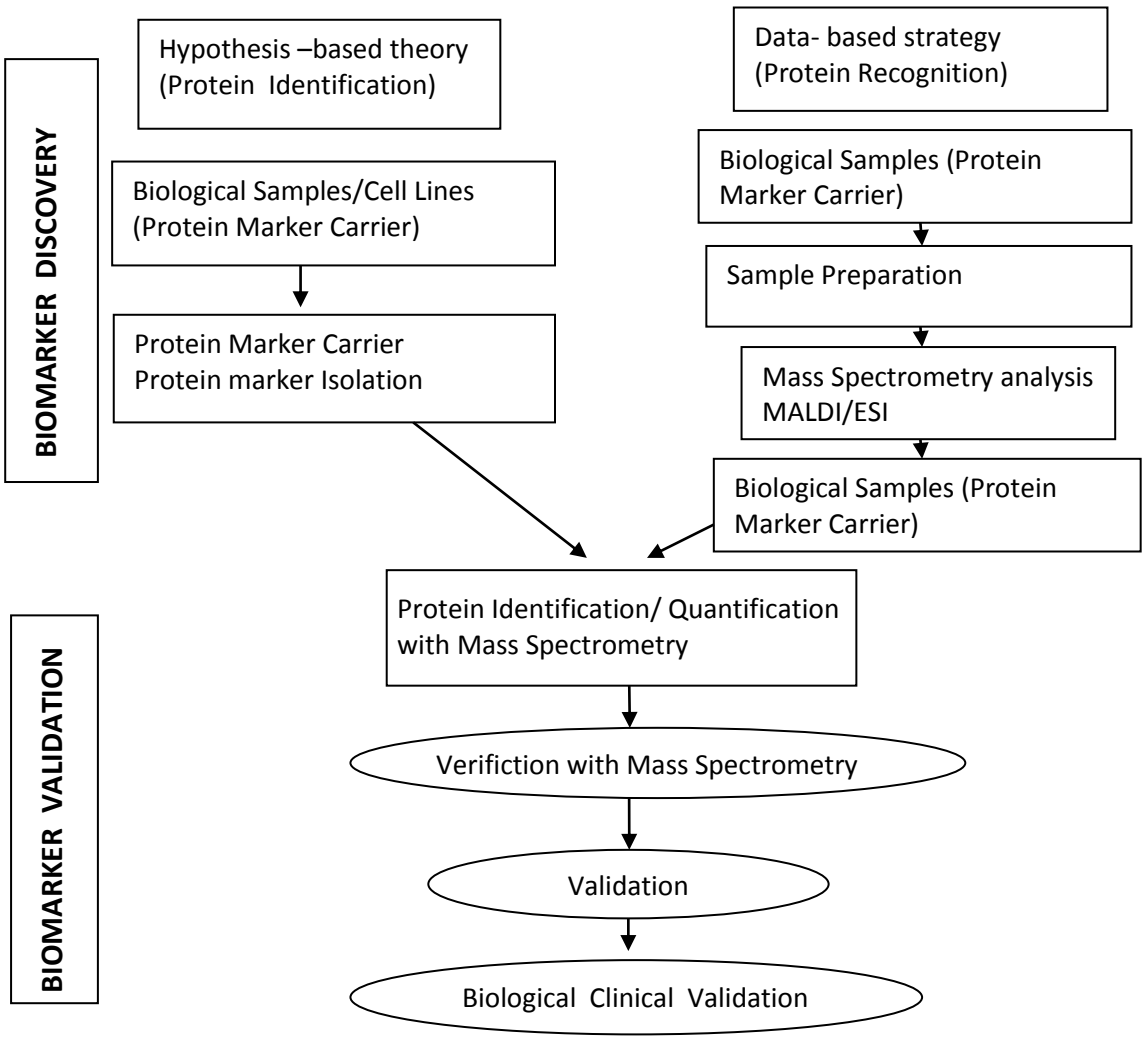

Figure 1: Workflow diagram of Maas Spectrometry based proteomics strategies and platforms used in cancer biomarker discovery and validation.[10]

PROTEOMIC BIOMARKERS

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.[11] 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.[12]

Over the past two decades, proteomics technology for cancer detection has attracted much research interest. Thus, much research effort has been dedicated to investigate potential biomarkers for oral cancer through proteomics. This review article highlights the potential biomarkers of oral cancer approached though salivary proteomics .

TABLE-1 Potential biomarkers of oral cancer

BIOMARKERS	REFERENCES
Mac- 2 binding protein, profilin,MRP14	13
IL-6, IL-8	14
CD-44	15
IL-4	16
Cofilin-1	17
b Fibrin	17
Transferrin	17
Transthyretin	17
MMP-2,9,3,10	4
Cytokeratin 19	4
Cyfra 21-1	4
tissue polypeptide antigen same	18
Cancer antigen 125	18
CD 59	13
Catalase	13

Proteomic application in systemic cancer

Proteomics technologies have experienced rapid advances over the last decade to identify or quantify thousands of proteins per sample, typically in a few hours, enabling proteomics applications in biological, medical, and clinical research. Today proteome profiling is extensively used for identification and monitoring of specific biomarkers in biological fluids as blood, cerebrospinal fluid, sputum and urine[19]as they contain proteins that can be

instructive for disease detection.[4]This has revolutionized the way of diagnosis, treatment and prevention of multi-factorial and toxicant borne diseases.[19]

Apart from oral cancer biomarker detection Proteomics has extended its network in other pathological diseases too. Till date, many research studies have been published and different biomarkers has been extracted through the proteomic technology.

Table 2 : Potential cancer biomarkers of other pathological diseases.

Types of Cancer	Biomarkers	References
Prostate	PSA	20
Breast	BRCA1,CA-15.3,LOX-12,CD24	21,22,23,24
Colorectal	CEA,VEGF,Transgelin-2 & ALCAM/CD	25,26,27,28
Pancreatic	AlCAM & hCG	29,30
Gastric	MG-7	31
Hepatic	AFP	32
Lung	proGRP	33
Ovarian	HE4	34

CONCLUSION:

In the field of oral cancer research salivary proteomics has declared hopes in identification of biomarkers for early detection and prognostication of oral cancer as well as pinpointing therapeutic targets for improved treatment outcomes. The application of established and novel proteomic technologies to the molecular analysis of cancer has a boundless future.

Although proteomics has demonstrated promise for biomarker discovery, further work is required to enhance the performance and reproducibility of established proteomics tools before they can be routinely used in clinical medicine. Improvements in data and bioinformatics tools, such as enhanced mass accuracy and resolution, faster protein identification algorithms, reduced false-positive rates and automated data validation strategies, will allow the assimilation of results across multiple studies and platforms, thus improving the depth of knowledge on a particular question [34]. With these improvements ambitious goals such as the creation of a full proteome map of tissues or cell types will be achieved more readily. Apart from analytical improvements, proteomics should be integrated with genomics, transcriptomics and metabolimics to reach the ultimate aim of systems biology, which is to further the understanding of complex biological systems. Therefore, proteomics should not be regarded separately, but rather as an important element of systems biology.

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