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Review Article

Human Salivary (Biomarkers) Proteome and Transcriptome: A Boon in Modern Cancer Diagnosis- A Systematic Review

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Abstract

Background: Over the last few decades our understanding of the molecular basis of tumour pathology has greatly improved and as science and technology is growing beyond mankind's yet explored fathoms, it is but imperative for us to utilize these new approaches to try and change the age-old methods of cancer diagnosis. Through these studies we have gained an insight into the world of molecular biomarkers and specific target therapies which are currently being developed. Moreover, the use of saliva as a bio-fluid for these studies has been significant to highlight the non-invasive approach to detect these biomarkers. The advancement of these omic studies have also called in for more specific and sensitive research techniques. The purpose of this review is to highlight the importance of salivary-biomarkers the proteomes and transcriptomes, in early cancer diagnosis and to pave the way for better conceptualising the ideologies of the proteomics and the transcriptomics world.

Aim of the Study: To determine the significance of Salivary Proteomes and Transcriptomes in the field of cancer diagnosis.

Research Question: Are salivary proteomes and transcriptomes significant in supporting early cancer diagnosis?

Materials and Methods: The study sample included review and research articles based on databases from the COCHRANE collaboration as well as few other scientific libraries like Medline and Med-know, having undergone a definite Randomized Control Trial (RCT), to signify the title of the study. Various inclusion and exclusion criterion for Systematic Review decided the final RCT based research articles for the study. (For the given study there were 65 articles which were selected out of which 45 were chosen post having undergone Randomised Control Trial).

Keywords: Oral Cancer; Proteomics; Transcriptomics; Saliva

Introduction

In today's day and age cancer is one of the leading causes of death in the world. It refers to an uncoordinated, uncontrolled and abnormal growth of cells which have either been subjected to genetic mutations or have undergone epigenetic alterations leading to an abnormal expression by those genes. Under these circumstances it is but imperative for us to study this crippling disease to understand its functional and polymorphic nature for its early diagnosis. The advent of Human Genome Project and dawning of microarray technology with the emergence in the field of bioinformatics has allowed us to foray into a new field of science the -omics study via understanding the biomarkers which they are constituted

of. While on one hand proteomic study allows us to understand the fundamental responses by a given cell to different stimuli allowing it to produce a spectrum of protein profiles providing direct information about cell signalling pathways and its general physiology. On the other hand Transcriptomic studies refer to the understanding of Transcriptome that is the study of a full range of RNA both the coding as well as the non-coding ones in form of transcripts and these transcripts provide useful information regarding the normal physiology of the cell and allows scientists to understand how changes in the normal amount of a gene activity may contribute to various disease pathologies.

Discussion

Literature states biomarkers as quantifiable biological parameters that can be measured or evaluated as an indicator to understand normal biological, pathogenic, or pharmacological responses to any given therapeutic intervention (National Institute of Health-NIH) [1]. As we humans navigate through these complicated times it is but imperative for us to try and reduce the global burden of diseases in every way possible. However, the general stigma associated with the diagnosis of the most prevalent diseases is the inability of these diseases to present themselves with specific symptoms during their early stages thereby reducing their chances of better prognosis. This is the very reason why conditions such as oral squamous cell carcinoma and many others as such are not able to be diagnosed unless they reach their advanced stages. However, in today's day and age, most of the current diagnostic methods lack easy-to-use inexpensive sampling methods which thereby could be accurately procured under a portable platform to facilitate better disease prognosis creating a need for more sustainable and practical diagnostic realms. As technology begun advancing so did our understanding of these complex macromolecules or the biomarkers. The modern era for cancer biomarker discovery didn't however begin up-until 1965 when Dr Joseph Gold found substances in the blood serum of the patients frequenting with colon cancer, a protein normally found in the fetal tissues he named it, Alfa-fetoprotein carcinoembryonic antigen or CEA [2,7] was discovered facilitating the introduction of immunological techniques such as "radioimmunoassay". It was then in the mid-1980s when hybridoma technology took over assisting in the discovery of Ovarian Epithelial Cancer Marker Carbohydrate Antigen or CA 125 [3,7] along with one of the best cancer bio-marker Prostate Specific Antigen or PSA KLK3.

It's evident that in every era of bio-marker discovery there have been close associations to its emergence of new and powerful analytical techniques. Although there is a huge surge in the discovery of biomarkers in saliva it is but imperative for us to understand that bio-molecular concentrations in saliva are but one-tenth to a thousandth of the levels found in the blood thereby leading to an urgent need for insensitive detection technology platforms.

Why is saliva considered to be a biomarker source?

Human saliva is a complex diagnostic fluid that is secreted mainly via the combinations of major and minor salivary glands [1]. It is a plasma ultra-filtrate containing proteins that have either been synthesized within the gland or have been derived via the blood [2]. The major protein biosynthetic mechanism for the salivary protein factory begins at the gene transcription and translation changes within the glands. These changes are followed by a number of post translational changes such as: - glycolyslation, acetylation, phosphorylation and proteolysis. Post these changes as the bio-fluid passes through the duct it undergoes further changes such as Proteolytic cleavage, partial deglycosylation, and protein-protein complex formation [3,4]. Hence it can be said that the saliva produced in the salivary gland is different from the saliva when secreted in the oral cavity, further upon release the saliva gets mixed with several non-exocrine, cellular and exogenous components, ultimately constituting something known as the "Whole Saliva".

The "Whole Saliva" is a mixture of saliva from a combination of major and minor salivary glands along with Gingival Crevicular Fluid, expectorated bronchial secretions, cells from oral wounds, micro-organisms, protein from food debris, and de-squamated epithelial cells. This goes on to show that the whole-saliva is highly variable and represents a complex balance between the local and the systematic factors. This notion/idea can be used into consideration while using saliva as a diagnostic fluid [2,5].

Human saliva provides multiple advantages over traditional biochemical analysis using blood. It provides and accounts for easy and stress-free sample collection, it accounts for non-invasive techniques, reduced need for free sample processing, minimal risk of contracting infectious organisms such as HPV, HIV, etc. It can also be a good source for being an ideal bio-fluid for third world countries as it is associated with low-cost sample collection [5-8].

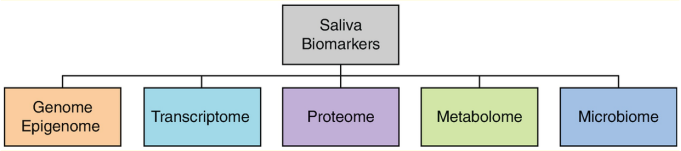


Figure 1: Biomarkers present in saliva.

Image source: Salivary Biomarkers in Oral Cancer: Oral Cancer Detection. Springer, Cham [6].

Human salivary proteomes

Human saliva constitutes of a huge collection of variegated proteins each with various and distinct functions. Although the content is estimated to be 30% of that of blood [9], saliva is a rich source of protein biomarkers which play a considerable role in digestion, maintaining homeostasis, lubricating oral fluid, defence mechanism and many more. Being capable of discerning healthy from diseased subjects, proteomes are proved to have discriminatory profiles for detection of diabetes, oral cancer, AIDS, periodontal disease and mammary gland carcinoma.

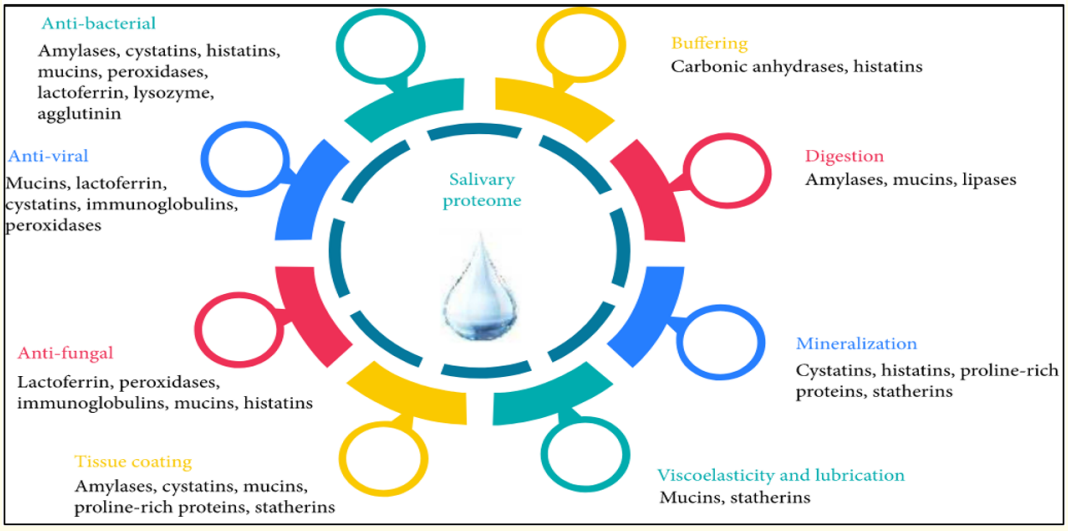


Figure 2: Biomarkers present in saliva.

Image source: Salivary Biomarkers in Oral Cancer: Oral Cancer Detection. Springer, Cham [6].

Proteomes being the most functional of products encoded by genes, have always facilitated in governing the biologic dictates of an organism by their structure and function. In conditions like carcinoma, proteomics encompasses the study of expressed proteins and elucidates the structural and functional relationship between the non-carcinogenic and carcinogenic conditions. Proteomic technologies are the valuable discovery in the field of biomarkers that help in identification of markers for cancer diagnosis, monitoring in disease progression and detection of therapeutic targets. A study by Yan., *et al.* [13] compared the salivary proteome to the plasma proteome and therefore documented a total of 1939 non-redundant proteins compiled out of 19474 unique peptide sequences from the whole as well as the ductal saliva while the plasma proteome yielded a net of 3020 proteins 597 of which were also found in the human saliva. Proteomic studies have catalogued 1166 proteins in major salivary gland secretions (914 were recovered from the parotid, 911 from the submandibular and the sublingual glands with 57% being common to both the glands [11-13].Even the salivary transcriptome (to be discussed later) has been brought to light using micro-array profiling with approximately 3000 mRNAs being found in the whole saliva. Several investigations have been attempted to use high technologies and current salivary proteomic and transcriptomic knowledge for biomarker discoveries, especially for cancer researches. At the same time techniques such as

LC, gel electrophoresis, nuclear magnetic resonance, immunoassay, and lectin probe analysis have been used to identify proteins present in the glandular saliva. In recent years the LOC (lab-on-chip) and POC (point-of-care) technologies have been developed to build automated, miniaturized, and multiplexed platforms for rapid assays. [15-16].Currently, Texas/Kentucky Saliva Diagnostics Consortium is at the forefront of developing 3-D bead saliva/oral fluid diagnostics for cardiovascular, cancer, and periodontal diseases [14,15].Given below is a small road map that helps us with the steps involved in the protein extracting mechanism.

Proteins map the phenotypic arrangement of the cells. They help identify the total number of genes in a genome which helps to put the pieces of the overall architecture of the cellular mechanism thereby giving an idea about the functional annotations of a given genome.

↓

Proteomics in turn gives a 3 – D model of every protein that explains more about their characteristics. As a result, proteomics can be used to allow the characterisation of post – transitional protein modification in the cell.

↓

Post – translational phase, proteins get modified in response to a variety of factors such as intra and extra cellular signals.

↓

These signals can associate themselves with a number of disease patterns in respect to their initial responses. For example, a particular kind of disease at its initial stages can relay certain characteristics instructions such as hormonal changes directed to a given particular cell. These signals when picked up by a cell can thereby be used as sources of post – translational modifications for that particular disease.

↓

Therefore, with this knowledge, proteomics can help us identify the subcellular locations of the proteins and tell us more about its function, and their interactions within the cell.

↓

The expression thus obtained after studying the proteome or sub – proteome can be compared and pitted against each other which would help us in identifying the disease specific protein.

The salivary proteomic model contains molecules that are synthesized by the human salivary glands and the proteins which cross the endothelium and the epithelium. While the lipophilic molecules including the steroidal hormones such as testosterone, oestrogens, and progesterone are transported into saliva via passive diffusion the other water and electrolytes pass into saliva via filtration processes through the pores in the acinar cells [17-19]. Along with the peptides in the blood which are transported via the protein channels, the large proteins are transported in the saliva via pinocytosis [4].

Human salivary proteomic analysis for cancer diagnosis

It has been widely appreciated that proteomic analysis of human whole-saliva for given disease-specific biomarker can be accepted to be a source of easy-to-use and a powerful diagnostic tool as well. When studied right it could easily define the onset, progression, and prognosis of human diseases especially in cases of benign and malignant tumours. In the same way, saliva profiling throughout disease progression could reveal potential biomarkers indicating different stages of diseases.

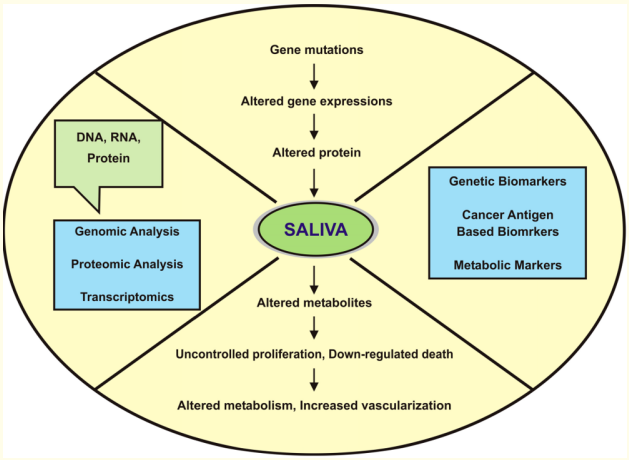
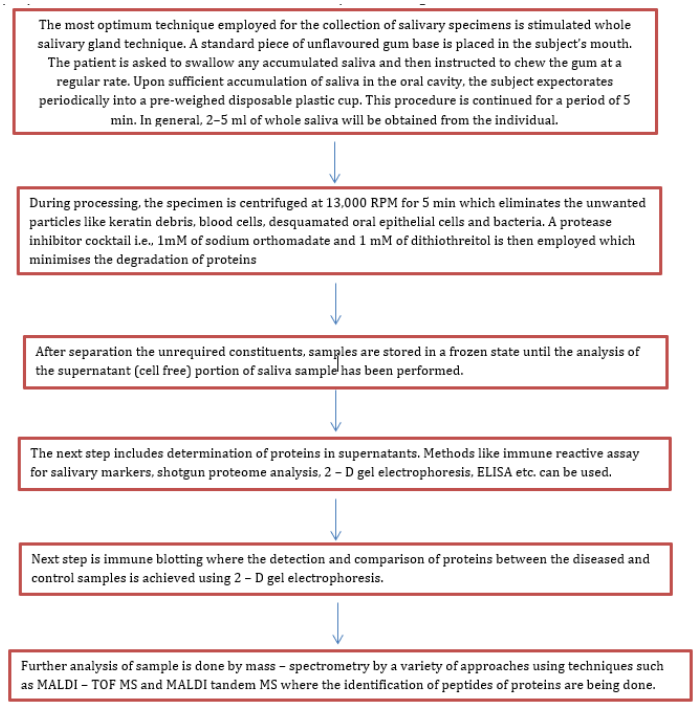


Figure 3: Clinical utility of saliva and the processes are showing carcinogenesis prospects for biomarkers.

Image source: Sannam Khan R., *et al.* "Advances of Salivary Proteomics in Oral Squamous Cell Carcinoma (OSCC) Detection: An Update". Proteomes 4.4 (2016): 41.

The field of salivary proteomics has efficiently expanded our knowledge about the composition of proteins in saliva. With the advent of 2D gel electrophoresis and mass spectrometry technology, the protein catalogue of saliva has expanded several hundredfold. This expansion has revealed proteins that reflect a myriad of functions that are altered in presence of disease [20]. Head and neck cancers are considered to be one of the most morbid human malignancies accounting for poor prognosis and severely compromised quality of life [21]. Most of these tumours are detected in the advanced stages due to a lack of efficient diagnosis. An efficient, rapid, and sensitive technique such as mass spectrometry can be used to detect the differentially expressed proteins as tumor-specific biomarkers in the whole saliva for head and neck squamous cell carcinoma patients. Several proteins such as the annexin A1, beta- gamma-actins, cytokeratin 4 and 13, zinc finger proteins, and

P53 [21] pathway proteins can be studied as these play a vital role in the tumor-genesis process. The major advantage of such a diagnosis is not only the results are easily reproducible but also reliable in cases of an early cancer diagnosis.



Salivary biomarkers could also be employed to study the progression of oral mucositis in cases of head and neck carcinoma patients. Biomarkers such as EGF (Epidermal growth factor), CRP (C-reactive proteins), TNF-alpha (tumor necrosis factor-alpha), and ESR or (Erythrocyte sedimentation rate) all can be employed to understand the progression, diagnosis, and monitor the prognosis in cases of Head and Neck carcinoma cases. Streckfus., *et al*. [22,23] measured soluble c-erbB-2Her2/neu levels saliva from breast cancer patients and concluded that the c-erbB might have potential use in screening, diagnosing, and determining the recurrence in cases of breast cancer. Similarly, salivary proteomics can be used to study the proteomic biomarker levels in cases of oral squamous cell carcinoma, ovarian cancer, salivary gland tumor, prostate adenocarcinoma, etc. CA-15-3 is one such protein tumor marker that is found in the saliva and could be potentially used to monitor advanced and metastatic cases in cases of breast cancer. Similarly, Fibroblast growth factor 2 (FGF2) and Fibroblast growth factor receptor 1 (FGFR1) concentrations are elevated in saliva in cases of patients with salivary gland tumors. The elevated levels of Prostate-specific antigen can be directly correlated with the detection of prostate adenocarcinoma [23,24].

Even in today's day and age clinicians lack tests that can accurately distinguish between a pre-malignant tumor and ones which have already progressed into malignancy [24]. It has been seen that bio-informatics analysis of exfoliated cells from the subject's saliva revealed an increase in myosin and actin in the malignant lesions also confirmed by western blotting. Nakashima et.al investigated and found out that in cases of adenoid cystic carcinoma 4-up regulated and 5-down regulated proteins are seen present. Some studies have revealed an important relationship between some proteins, such as transketolase, dim 1 p, v- ha-ras oncogene type I collagen pro alpha, tumor necrosis factor (ligand) superfamily member 4, Pirin, and tumor metastasis. Some studies have also investigated the differential expression of proteins in adenoid cystic carcinoma with lung metastasis and found that transketolase with modulator recognition factor 2, dim1p homolog, splicing factor (arginine/serine-rich 9) and v-ha-ras 1 oncogene was less expressed in poorly metastasized tumours and significantly up-regulated in highly metastasized tumours [24]. Currently, as of now, there are more than 100 recognized biomarkers used for the detection of oral cancer which includes nucleic acids and proteins. In particular, in cases of oral squamous cell carcinoma patients there are statistical evidence of significant changes in the level of salivary transcriptomics for instance: - IL-8, CD44 (a cell surface glycoprotein used for the cell-to-cell interaction), MMP-1, MMP-3, and recently Cyfra 21-1 (fragment of cytokeratin-19) and ZNF510 [11,25-27]. Studies have also shown how ZNF510 has been used to study the differences in early and late tumours especially in cases of oral squamous cell carcinoma cases. Exosomes and micro vesicles (MV's) of Head and neck carcinoma patients have been seen to be positively related to the progression in cases of oral squamous cell carcinomas [28]. However, one must also understand that poor design and quality of scientific studies in cases of oral cancer biomarkers indicate a lack of evidence used for the salivary biomarkers in oral cancer diagnosis. Even in studies, there is a lack of consensus and methodological criterion in cases of marker choices.

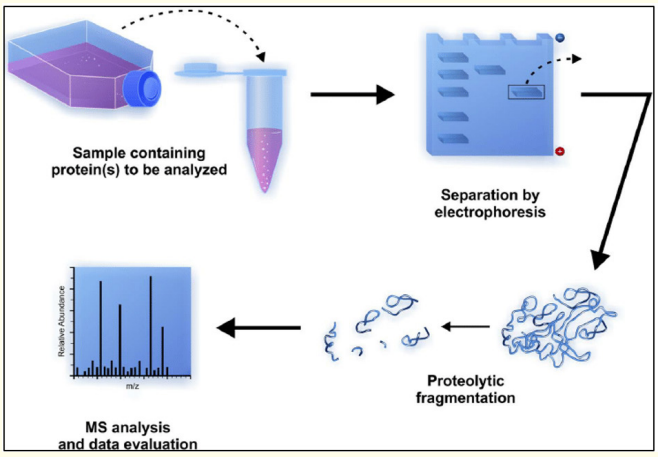


Figure 4: Steps of proteomic analysis via mass-spectrometry after separation and in-gel digestion.
Image Source: János András, Mótyán, Ferenc Tóth and József Tőzsér *et al*. Research Applications of Proteolytic Enzymes in Molecular Biology.

Human salivary transcriptomic analysis for cancer diagnosis

The term Transcriptome is a portmanteau that includes the words "TRANSCRIPTS" and "GENOME" and is directly associated with the idea of transcription [29]. The transcriptome is a collection of all RNA transcripts including the coding and non-coding transcripts in a given population of cells. It finds its etymological origins in the formation of cDNA libraries, the first of which was published in silk moths around the year 1979, and a seminal study of this was published in the year 1997 [30-31]. As Bioinformatics flourished over the year so did our understanding of-omics and -home studies. The central dogma [32] of molecular theory presents transcriptome as a composition of protein-coding mRNA transcripts followed by several RNA subtypes with given distinct features. Now, this is applicable as transcriptome contains significant information of all the RNA transcribed by the genome under given specific physiological or pathological conditions. Thereby allowing us to understand the human genome at the very transcriptional level and successfully disclosing comprehensible gene structure. A significant analysis could be made in respect to understanding key alteration processes triggering human diseases, thereby offering instrumentation to comprehend their underlying mechanisms and improve molecular diagnosis and clinical therapy. The very fact that human mRNA [34] is present in the saliva is significant enough to understand the importance saliva has to understand translational and diagnostic analytics during disease diagnosis. One must understand that RNA is more reliable than DNA but more susceptible to degradation via RNAses [34,35]. It has been commonly presumed that human RNAase activity is elevated in patients who have cancer thereby presuming that human mRNA cannot survive extracellularly from the saliva [36]. For a normal healthy individual, the source to detect human salivary RNA could be one of three major salivary glands, gingival crevicular fluid [37], or oral mucous cells lining or desquamated. Currently, many types of research are being carried out to obtain stratified oral fluids from these sources to reconstruct salivary transcriptome in normal subjects. For instance, in an oral cancer patient, the associated RNA signature for a given detected tumor is likely to be originated from a matched tumor or a systemic response (local or distant) that further reflects itself in the whole saliva from either of the sources mentioned above. It has been understood that a disease associated RNA can easily find its way through into the oral cavity/fluid via the gingival crevicular fluid. One such good example is the presence of HER-2 proteins in the saliva for a breast cancer patient [38].

For any instances of oral cancer, the local tumor is the source of elevated mRNA. Early analysis has supported the data matching the tumor source of oral cancer salivary via the RNA signature. On using restricted microarray (HG-U95A, Affymetrix, 12,167 probes) [39] for previous oral tumor expression studies, IL8, IL1B and ferritin polypeptide mRNAs were found to be elevated significantly in the saliva of the oral cancer patients and the surrounding tumor affected tissue. Chen., *et al*. has independently demonstrated the elevation of IL-8 expression in the patients frequenting neck cancer tissues [33,36,40]. In addition to IL-8, other six cancer-associated genes have shown an increasing tendency in the saliva of the patients having oral cancer - these being: DUSP, H3F3A, OAZ1, SAT, S100P, and IL1B. Some of them such as the DUSP1 gene encodes a dual-specificity phosphatase and has been presented as a mediator for tumor suppressor signaling pathway. DUSP1 gene's expression has been shown to decrease in cases of ovarian tumors. H3F3A mRNA is used as a proliferative marker whose levels are up regulated in conditions of prostate cancers and colon cancers. OAZ1 is said to be a tumor suppressor based on its inhibitory functional abilities to prevent the action of ornithine decarboxylase, however,

it has been interestingly noted that OAZ1 mRNA is up-regulated in cases of prostate cancer [36-41]. Precision cancer therapies exploit the ideologies of the tumour-patient specific molecular features to manipulate the tumour micro-environment. However one knows as a matter of fact that the diversity within a single cell tumour microenvironment transforms this complicated fact.

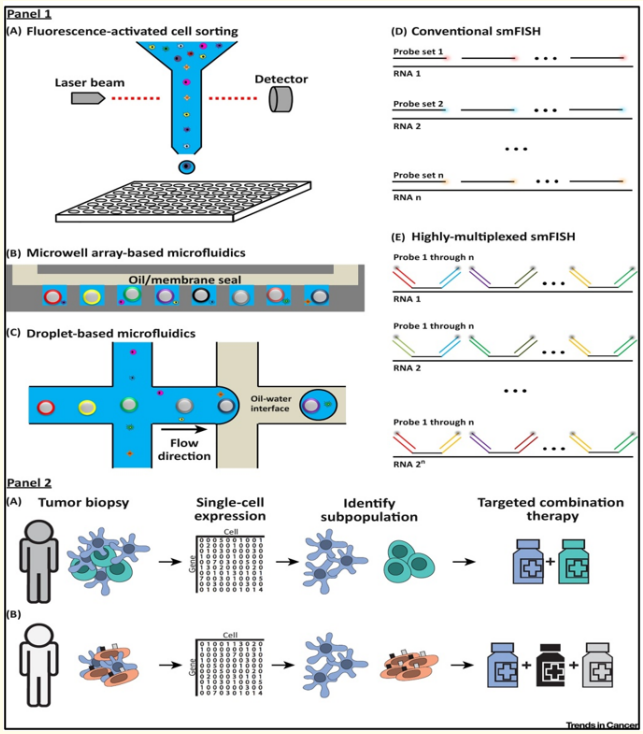


Figure 5: Applications of Single cell Transcriptomics in Precision medicine.

Image Source: Levitin, Hanna Mendes *et al.* Single-Cell Transcriptomic Analysis of Tumor Heterogeneity". Trends in Cancer 4.4 264-268.

SAT another identified mRNA that is mainly involved with polyamine metabolism is also said to be up regulated in cases of prostate cancer [42]. S100 defines prostate cancer progression and its overexpression is associated with the early stages of breast cancer development. On the other hand, S100P is associated with instances of pancreatic cancer. The expression of IL1B [43] is higher in the ascetic fluid of women with ovarian cancer and genetic polymorphism of IL1B has been reported to have potential associations with gastric cancers and breast cancers.

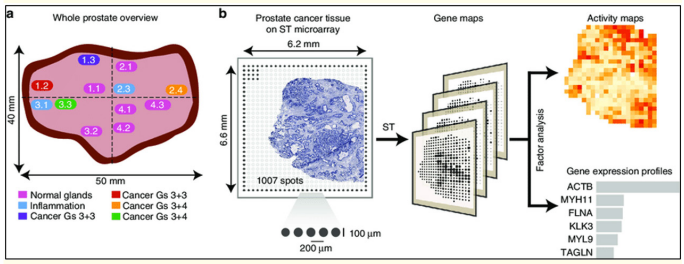


Figure 6: Study design in regards to the concept of spatial transcriptomics in a given case of prostate cancer. A. Location of the annotations as guided by the pathologist, in this case the different colours are represented in different annotations. The scale bars are indicative of the size of the area within the prostate referred to. B. Presence of microarrays representative of the spatial form have 1007 barcoaded spots of each 100 µm diameter further with 200 µm centre-to-centre distance. Dark spots denoted by points and circles lack the spatial barcodes.

Image source: Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity". Nature Communications [45].

The biological importance of how differentially these genes express in various cases of head and neck cancers and other cancers must be determined. Identification of cancer-associated genes that remain consistently changing in cases of cancer patients would not only help us to understand the associated diagnostic marker for that particular tumor but also would provide an insight into the associated molecular profile involved in head and neck cancer progression [44]. Understanding the correlated molecular trend would be extremely useful as it would help us to possibly match the resulting phenotype of the given cancer with its molecular events. The given multifactorial nature of oncogenesis and the described heterogeneity seen in the oncogenic pathway makes it unlikely that a given single biomarker would be able to detect all tumors with utmost specificity and sensitivity. Therefore this idea calls for more specific researches and discoveries to understand the very fundamental idea of tumor condition [45,46].

Conclusion

Currently, saliva is being considered to be used as an investigational aid in the diagnosis of systemic diseases such as HIV infec-

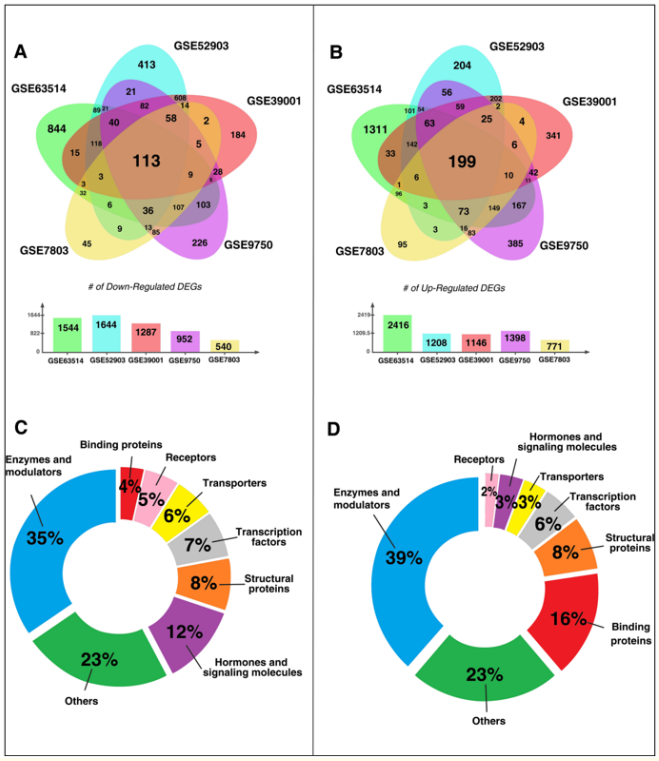


Figure 7: Meta-analysis of a given transcriptomic dataset for cervical cancer. A. Venn diagram showing the distribution of down-regulated transcripts found in the given dataset. B. Venn diagram showing the distribution of up-regulated transcripts in the datasets. C. Cluster of proteins as a result of down-regulation in the core genes of cervical cancer in accordance to their molecular activity. D. Clustering of proteins in reference to being up-regulated in the core genes of cervical cancer in accordance to their molecular activity.

Image source: Kori M and Yalcin Arga K. "Potential biomarkers and therapeutic targets in cervical cancer: Insights from the meta-analysis of transcriptomics data within network biomedicine perspective". PLoS ONE 13.7 (2018): e0200717.

tion, diabetes mellitus, periodontal cases, dental caries, etc. Moreover, a better understanding of the complex macromolecular world of biomarkers could help us to understand the disease progression and allow us to monitor them for ensuring a better prognosis. Although most of the above-mentioned ideologies are in their nascent stages, specific modifications are necessary to be addressed for the acute development of the salivary proteomic and transcriptomic studies. Now, this field is essentially crucial as not only do they help in monitoring the early occurrence of the tumors, but also allows one to understand the complex processes of cancer coercion to its malignant form. Therefore, as we look towards the future and hope for better diagnostic, achievements, it is but imperative for us to carefully analyse and understand the significance of these macromolecules, for it is their modifications and dynamics which would help us to introduce saliva into the field of clinical laboratory, as effective biological fluid for disease prognosis and tumor diagnosis.

Bibliography

1. Institute Of Medicine (US) Forum on Drug Discovery, Development, And Translation. "Accelerating The Development of Biomarkers for Drug Safety". Workshop Summary. Washington (DC): National Academies Press (US) (2009): 1.

2. David T Wu., *et al*. "Saliva - A Promising Tool for Diagnosing Oral Diseases". *Current Oral Health Reports* 5.1 (2018).

3. Helmerhorst EJ., *et al*. "Saliva: A Dynamic Proteome". *Journal of Dental Research* 86 (2007): 680-693.

4. Walsh CT., *et al*. "Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications". *Angewandte Chemie International Edition* 44 (2005): 7342-7372.

5. Benjamin L Schulz., *et al*. "Saliva Proteome Research: Current Status and Future Outlook". *Critical Reviews in Biotechnology* 33.3 (2013): 246-259.

6. Panta P., *et al*. "Salivary Biomarkers in Oral Cancer". In: Panta P. (eds) Oral Cancer Detection. Springer, Cham (2019).

7. Gold P and Freedman SO. "Demonstration of Tumor-Specific Antigens In Human Colonic Carcinomata By Immunological Tolerance And Absorption Techniques". *Journal of Experimental Medicine* 121 (1965): 439.

8. Pfaffe T., *et al*. "Diagnostic Potential of Saliva: Current State and Future Applications". *Clinical Chemistry* 57 (2011): 675-687.

9. Schulz BL., *et al*. "Saliva proteome research: current status and future outlook". *Critical Reviews in Biotechnology* 33 (2012): 246 -259.

10. A Van Nieuw Amerongen., *et al*. "Salivary proteins: protective and diagnostic value in cariology?" *Caries Research* 38.3 (2004): 247-253.

11. David T Wu., *et al*. "A Promising Tool for Diagnosing Oral Diseases". *Current Oral Health Reports* 5.1 (2018).

12. Yan W., *et al*. "Systematic Comparison of The Human Saliva and Plasma Proteomes". *Proteomics - Clinical Applications* 3 (2009): 116-134.

13. Christodoulides N., *et al*. "Lab-on-A-Chip Methods for Point-of-Care Measurements of Salivary Biomarkers of Periodontitis". *Annals of the New York Academy of Sciences* 1098 (2007): 411-428.

14. Lee YH., *et al*. "Saliva: An Emerging Biofluid for Early Detection of Diseases". *American Journal of Dentistry* 22.4 (2009): 241-248.

15. Kathariya R., *et al*. "Salivary Proteomic Biomarkers for Oral Diseases: A Review of Literature". *AOSR* 1.1 (2010): 43-49.

16. Gröschl M., *et al*. "Current Status of Salivary Hormone Analysis". *Clinical Chemistry* 54 (2008): 1759-1769.

17. Liu C., *et al*. "A Timer-Actuated Immunoassay Cassette for Detecting Molecular Markers in Oral Fluids". *Lab on a Chip* 9.6 (2009): 768-776.

18. Pfaffe T., *et al*. "Diagnostic Potential of Saliva: Current State and Future Applications". *Clinical Chemistry* 57 (2001): 675-687.

19. Charles F Streckfus., *et al*. "Proteomic analysis of saliva for cancer diagnosis". *Expert Review of Proteomics* 4.3 (2007): 329-332.

20. Jarai T., *et al*. "Mass Spectrometry-Based Salivary Proteomics for The Discovery of Head and Neck Squamous Cell Carcinoma". *Pathology Oncology Research* 18.3 (2012): 623-628.

21. Streckfus C., *et al*. "The Presence of Soluble C-Erbb-2 In Saliva and Serum Among Women with Breast Carcinoma: A Preliminary Study". *Clinical Cancer Research* 6 (2000): 2363-2370.

22. Streckfus CF., *et al*. "Breast Cancer Related Proteins Are Present in Saliva and Are Modulated Secondary To Ductal Carcinoma In Situ Of The Breast". *Cancer Investment* 26 (2008): 159-167.

23. Yogesh Goswami., *et al*. "Biomarkers-A Review of Powerful Diagnostic Tool". *IOSR Journal of Dental and Medical Sciences* 14.3 (2015): 80-87.

24. Fabryovah Celec P., *et al*. "On The Origin and Diagnostic Use of Salivary RNA". *Oral Diseases* 20.2 (2014): 146-152.

25. Gualtero DF., *et al*. "Biomarkers in Saliva for the Detection of Oral Squamous Cell Carcinoma and Their Potential Use for Early Diagnosis: A Systematic Review". *Acta Odontologica Scandinavica* 74.3 (2016): 170-177.

26. Panta P., *et al*. "Salivary RNA Signatures in Oral Cancer Detection". *Analytical Cellular Pathology* (2014): 7-17.

27. Principe S., *et al*. "Tumorderived Exosomes and Microvesicles in Head And Neck Cancer: ImplicationsFor Tumor Biology and Biomarker Discovery". *Proteomics* 13.10-11 (2013): 1608-1623.

28. Wikipedia Contributors. Transcriptome. In Wikipedia, The Free Encyclopedia.

29. Amelia Casamassimi., *et al*. "Transcriptome Profiling in Human Diseases: New Advances and Perspectives". *International Journal of Molecular Sciences* 18.8 (2017): 1652.

30. Yang Li., *et al*. "Salivary Transcriptome Diagnostics for Oral Cancer Detection". *Clinical Cancer Research* 10.24 (2004): 8442-8450.

31. Zhou AQ., *et al*. "Exploration of the central dogma at the interface of chemistry and biology: 2010 Yale Chemical Biology Symposium". *Yale Journal of Biology and Medicine* 83.3 (2010): 131-133.

32. Sannam Khan R., *et al*. "Advances of Salivary Proteomics in Oral Squamous Cell Carcinoma (OSCC) Detection: An Update". Proteomes 4.4 (2016): 41.

33. Park NJ., *et al*. "Characterization of RNA in saliva". *Clinical Chemistry* 52.6 (2006): 988-994.

34. Bechhofer DH., *et al*. "Bacterial ribonucleases and their roles in RNA metabolism". *Critical Reviews in Biochemistry and Molecular Biology* 54.3 (2019): 242-300.

35. Jarrous N., *et al*. "Human RNase P: a tRNA-processing enzyme and transcription factor". *Nucleic Acids Research* 35.11 (2007): 3519-3524.

36. Barros SP., *et al*. "Gingival crevicular fluid as a source of biomarkers for periodontitis". *Periodontology 2000* 70.1 (2016): 53-64.

37. Lei B., *et al*. "Transcriptome sequencing of HER2-positive breast cancer stem cells identifies potential prognostic marker". *Tumor Biology* 37.11 (2016): 14757-14764.

38. Hackstadt Amber and Hess Ann. "Filtering for Increased Power for Microarray Data Analysis". *BMC Bioinformatics* (2009). 10-11.

39. Aggarwal BB., *et al*. "Inflammation and cancer: how friendly is the relationship for cancer patients?" *Current Opinion in Pharmacology* 9.4 (2009): 351-369.

40. Rabadán R., *et al*. "Identification of relevant genetic alterations in cancer using topological data analysis". *Nature Communications* 11 (2020): 3808.

41. Gornati R., *et al*. "Evaluation of SAT-1, SAT-2 and GalNAcT-1 mRNA in colon cancer by real-time PCR". *Molecular and Cellular Biochemistry* 298.1-2 (2007): 59-68.

42. Yin Shenglu., *et al*. "Expression of interleukin 1β in gastric cancer tissue and its effects on gastric cancer". *OncoTargets and Therapy* 9 (2015): 31-35.

43. Belbin TJ., *et al*. "Molecular Profiling of Tumor Progression in Head and Neck Cancer". Archives of Otorhinolaryngology-Head and Neck Surgery 131.1 (2005): 10-18.

44. Berglund Emelie., *et al*. "Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity". *Nature Communications* 9.

45. Levitin Hanna Mendes., *et al.* "Single-Cell Transcriptomic Analysis of Tumor Heterogeneity". *Trends in Cancer* 4.4 264-268.

46. Kori M., *et al*. "Potential biomarkers and therapeutic targets in cervical cancer: Insights from the meta-analysis of transcriptomics data within network biomedicine perspective". *PLoS ONE* 13.7 (2018): e0200717.

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