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Review Article

Saliva as a sample for liquid biopsy: A review

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ABSTRACT

This paper reviews the recent advancements in liquid biopsy, focusing on its potential application in oral squamous cell carcinoma. Liquid biopsy, a non-invasive technique, offers a promising alternative to traditional biopsy methods by analyzing circulating tumor cells, exosomes, and circulating tumor DNA in bodily fluids like blood and saliva.The study highlights the advantages of saliva as a liquid biopsy substrate, including its ease of collection, non-invasive nature, and the presence of valuable biomarkers. Several potential markers for OSCC detection in saliva are discussed, such as somatic mutations, viral DNA, microRNAs, and protein biomarkers.While liquid biopsy holds great promise, challenges remain in terms of sensitivity, specificity, and standardization of techniques. Further research is needed to develop more reliable and cost-effective methods for analysing these biomarkers and translating them into clinical practice. By overcoming these limitations, liquid biopsy can revolutionize the early detection, diagnosis, and monitoring of OSCC, leading to improved patient outcomes.

1. Introduction

Recent developments in the field of oncology have advanced the concept of 'precision medicine', which is based on the idea that therapies should be tailored to the individualized patterns of a tumor. The Human Genome Project (HGTP) and pharmacogenomic research have revolutionized the field, providing a wealth of molecular data.[1] As a result, novel biotechnology has highlighted the limitations of traditional sampling methods, such as the invasive nature of biopsies and surgical procedures, the potential for complications, and the inability to perform these procedures when clinical conditions have worsened or a tumor is in inaccessible area. Furthermore, biopsy tissues provide a single-point in time image of the tumor, as well as the genetic heterogeneity of multiple tumor subclons.[2] Numerous studies have demonstrated that the genomic landscape of tumours and metastases dynamically evolves over time, responding to selective pressure from therapies that can inhibit or promote the growth of various cell clones. These limitations are especially evident when an acquired resistance to treatment is observed during follow up.[3] As research advances, we can anticipate saliva-based tests for a wide range of diseases, including cancer, infections, and neurological disorders. This could lead to earlier diagnosis, more frequent monitoring, and personalized treatment plans, ultimately improving patient outcomes.

2. What is Liquid Biopsy?

Recent research in oncology has focused on the components of cancer that are released into the bloodstream by apoptotic or necrotic cells. These components are referred to as circulating tumor DNA (ctDNA) and exosomes. Exosomes are membrane-secured subcellulars that contain proteins

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as well as nucleic acids that are released by tumor cells. Additionally, primary and metastatic tumour sites are capable of exfoliating vital cells that enter the bloodstream as circulating tumor cells.

This new diagnostic tool, known as 'liquid biopsy', is a solution to the initial limitations of the lack of nucleic acids and the difficulty of distinguishing between normal and tumor nucleic acids. Next Generation Sequencing (NGS) techniques have improved the sensitivity of these components, allowing for the detection of genetic and epizootic aberrations.[4]

3. Circulating Tumor Cells in Oral Squamous Cell Carcinoma

The release of circulating tumor-derived cells (CTCs) from the primary tumor is a result of either spontaneous or iatrogenic factors, and they are thought to have a similar profile to those present in the somatic and genomic alterations of the primary tumor.[5] CTCs are able to accurately reflect the heterogeneity of the tumor, which can be overlooked in invasive tissue biopsies, making them an ideal tool for understanding the mutational profiles of tumors without the need for patient biopsy. One of the major limitations of CTC's use in patient management is their limited presence in peripheral circulation, hence requires enrichment and separation through tedious and costly methods.[6,7] It has been observed that increasing CTCs levels have been linked to poor prognosis, as well as distant metastasis, in various forms of cancer. The survival rate of Oral Squamous Cell carcinoma (OSCC), as well as other types of cancer, such as those in breast, lungs, prostate, and ovarian, has been associated with elevated CTC levels. It was found that there is a high risk of loco-regional recurrence and distant metastasis associated with the presence of CTCs.[8]

There are multiple techniques available to detect CTCs. AdnaTest utilizes antibody-covered beads that are tailored to the type of cancer, and a real-time polymerized chain reaction is conducted to identify expression patterns on the cells. MACS (Magnetic-activated Cell Sorting) is a method of cell sorting that involves the addition of magnetic nanoparticles to the cells, and MagSweeper is a method of immunomagnetic enrichment that utilizes a robotic, ally-controlled magnetic rod and antibody-covered magnetic beads to separate CTCs from other blood cells after centrifugation. Target Selector, a CTC platform developed by Biocept, San Diego, California, USA; utilizes an antibody cocktail to detect CTCs that target Epithelial cell adhesion molecule (EpCAM) cells, as well as additional mesenchymal, stem cell, tumor-associated, and cell type-specific biomarkers at the protein and DNA levels of the microfluid channels.[9]

4. Exosomes in Oral Squamous Cell Carcinoma

Exosomes, tiny bioactive vesicles measuring 40-150 nm in diameter, are employed to analyze lymphoblastoid cells. A miRNA expression profile has associated circulating exosomal miR-21 with hypoxic tumor growth and lymph node metastasis in OSCC patients. The detection of miRNAs in both plasma and tumor tissue of SCCL patients underscores the potential diagnostic utility of free and exosomal miRNAs for tongue cancer. Encased within protein complexes or microvesicles, these miRNAs are shielded from blood RNAse activity, offering a more robust method to assess circulating tumor-iRNA profiles. Exosomes within the tumor microenvironment have been implicated in elevated TGF-A pathway activity, leading to increased drug resistance and tumor growth in OSCC. OSCC cell-derived exosomes, including those containing CMTM6, contribute to M2 macrophage polarization by activating ERK1/2 signaling pathways in macrophages.[10]

5. Saliva as a Liquid Biopsy Substrate

Saliva has been identified as a source of various inflammatory biomarkers, both for oral and systemic diseases, and has been used to detect a wide range of molecular markers for a variety of diseases, including cancer, heart disease, cardiovascular disease, and HIV. Since its inception in the early 1960s, the field has expanded to include detection of illicit drugs and alcohol in human saliva, the measurement of hormone levels, particularly estrogen levels, in women with hormone imbalances, and the diagnosis of HIV virus in HIV-infected patients. Development of more sensitive detection techniques in the field of Saliva Diagnostics has the potential to revolutionize the way diseases are diagnosed and clinically monitored.[11] Saliva contains adequate amounts of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and disease biomarkers, and collecting saliva is a simple, safe, and non-invasive process, hence it is a great substitute for serum in diagnostic procedures. Saliva does not clot, hence fewer manipulations are needed during diagnostic procedures, making it easier to handle than blood. It is one of the simplest ways to collect body fluids and only requires a chairside, non-invasive procedure without the need for specialized equipment. It makes extensive and frequent sampling possible in brief bursts of time. Saliva is recognized as a possible diagnostic tool in light of these.[12,13]

6. Markers for OSCC in Saliva

Saliva, plasma, and other body fluids contain somatic mutations of tumor-specific DNA, which are linked to the initiation and progression of cancer. The diagnosis of oral or other tumors can be made using these somatic mutations as biomarkers. All patients with oral tumors had positive

tumor-specific DNA in their saliva. Cristaldi M et al proved that, Saliva samples from patients with tumors in other parts of the body only contain tumor-specific DNA in 47–70% of cases. On the other hand, 86%–100% of patients with tumors in other sites and 80% of plasma samples from patients with oral tumors had tumour-specific DNA. These findings indicate that saliva contains an excess of DNA specific to oral tumors.[11] Thus, saliva containing DNA specific to tumors may be used to diagnose oral cancers. Human herpes virus (HHV) and HIV are two examples of tumour-related viruses whose DNA can be found in saliva and are linked to oral cavity and other cancers. According to Cheng, J et al., the levels of miR-125a and miR-200a were highly significant because they were found to be lower in the saliva of patients with oral squamous cell carcinoma (OSCC) than in healthy individuals. This suggests that salivary miRNAs may be useful in the detection of oral cancer.[12]

Tumor-specific genetic markers, found in both DNA and RNA, can be identified in saliva to detect oral cancer. These markers reflect the genetic alterations that drive tumor development and progression. Such alterations include somatic mutations in genes like p53 and tumor suppressors, changes in microsatellite sequences, abnormal promoter methylation, mutations in mitochondrial DNA, and the presence of tumor-associated viral DNA.[13]

A loss of genomic material in one of the chromosomal pairs is defined as loss of heterozygosity (LOH). Adeola, H.A et al., and Zhang L et al., have demonstrated that LOH is an early predictor of the malignant transformation of precancerous lesions in areas where a known human suppressor gene is present.[13,14] Califano J et al., have revealed that chromosomes 3p, 9q, 13q, and and 17p as a precursory event in the genesis of oral cancer. Mutations in mitochondrial DNA have also been helpful in find the salivary exfoliated OSCC cells. Such changes found in 67% of the saliva samples taken from the patients with OSCC using direct sequencing. The primary chromosome of the P53 gene is 17p. The arrest of the cell cycle and the start of the apoptosis in reaction to the damage to the DNA.[15] Liao PH et al., discovered the p53 mutation in the DNA taken from the OSCC patients' saliva, indicating a possible use as a biomarker to detect oral cancer. The research focused on mutations in p53 exon 4 codon 63, which was considerably great.[16]

Several genes in head and neck cancer have been found to exhibit promoter hypermethylation. For instance, Rosas et al. discovered aberrant methylation in at least one of three genes: p16, MGMT, or DAP-K, in oral squamous cell carcinoma (OSCC).[17] Zhong et al., investigated telomerase activity in the saliva of OSCC patients. They found that 75% of the cases exhibited telomerase positivity, suggesting that this could be a potential additional marker for OSCC detection.[18]

Li et al. utilized microarray analysis to examine the salivary transcriptome of OSCC patients. They identified seven markers that were significantly elevated in the saliva of these patients. Based on the magnitude of upregulation, these seven genes were categorized into three ranks: highly upregulated (IL-8), moderately upregulated (H3F3A, H3 histone family 3A, IL-1-β, S100P, and DUSP1), and lowly upregulated (OAZ1 and SAT).[19]

Using a thorough analysis of the human saliva proteome, Hu et al., found that the oral cancer patients' salivary proteins varied in terms of Mac-2 binding protein, myeloid related protein 14, CD59, profilin 1, and catalase. According to prognosis prediction, a number of salivary protein markers in the OSCC have been studied and have demonstrated relatively moderate sensitivity and specificity values. To fully understand the mechanism of action in a clinical setting, the biology underlying these promising targets requires further investigation. OSCC is often diagnosed at advanced stages, leading to poor prognosis and survival rates. Early detection is crucial for successful treatment and improved survival outcomes. Given that 10% of the general population exhibits oral mucosal abnormalities and precancerous or early cancerous lesions often lack distinct clinical characteristics, visual inspection alone may not be sufficient for early detection. Integrating early detection and screening methods based on salivary protein biomarkers with conventional oral examinations is essential. To identify discriminatory biomarkers for true early detection, retrospective proteomic analyses of oral precancer and cancer are warranted.[20]

7. Conclusion

Saliva offers numerous advantages as a diagnostic fluid, including ease of collection, convenient storage, non-invasive nature, and high-quality DNA content. These characteristics make saliva a suitable alternative to blood. Salivaomics research plays a crucial role in identifying disease biomarkers and potential drug targets. It also holds the potential for early-stage disease diagnosis. However, the field of saliva research and its applications in disease diagnosis is still in its early stages, hindered by the lack of efficient and cost-effective methods and techniques. Developing standardized salivary evaluation systems and molecular identification methods is essential. Building systemic knowledge networks of salivaomics and identifying precise disease biomarkers can contribute to a better understanding of the link between oral health and systemic health. This knowledge can facilitate the application of precision medicine by enabling individualized, precise, pain-free, and convenient targeted therapy.

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9. Conflict of Interest

None.

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