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Advancing Oral Cancer Diagnostics through Liquid Biopsy: A Review of Emerging Evidence

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Abstract

Oral cancer, a malignant growth originating in the tissues of the oral cavity, ranks as the eighth most common cancer globally. Its high mortality rate is largely attributed to diagnoses occurring at advanced stages. Although histological examination remains the gold standard of diagnostic, it necessitates invasive tissue sampling. In contrast, liquid biopsy has recently gained attention as a non-invasive and promising alternative for the diagnosis of oral cancer. This method analyzes tumor-derived materials and their surrounding microenvironment using biological samples such as blood, saliva, urine, and breast milk. Key molecular markers analyzed in liquid biopsies include circulating tumor DNA (ctDNA), exosomes, circulating tumor cells (CTCs), microRNAs (miRNAs), and cell-free DNA (cfDNA). Liquid biopsy offers significant potential for early detection, tumor molecular profiling, treatment monitoring, and identifying minimal residual disease. This narrative review explores the role of liquid biopsy in the diagnosis of oral cancer, highlighting its molecular targets, benefits, and clinical implications.

Keywords: Oral cancer, Liquid biopsy, Circulating tumor cells, cfDNA, miRNA

Introduction

Oral squamous cell carcinoma, commonly referred to as oral cancer, is the most frequent malignancy affecting the head and neck region. It often originates in areas such as the tongue, lips, and floor of the mouth [1]. Alarmingly, the global status report on oral cancer highlights a particularly severe burden in the Southeast Asian region [2], with India alone responsible for 36% of newly diagnosed cases and 42% of global oral cancer-related deaths. Despite advancements in treatment strategies, survival rates remain disappointingly low. Consequently, the development of rapid, accurate, and non-invasive diagnostic tools for early detection is critically important [3].

Several screening tools have been developed, including devices that function based on autofluorescence and spectrophotometry. Additionally, toluidine blue vital staining has been investigated for its potential in mass screening of early lesions. However, these optical methods often face limitations due to the complexity of result interpretation and a higher likelihood of false-positive outcomes [4].

Saliva, often described as a reflection of oral health, holds significant diagnostic potential. It contains a variety of biomolecules that can serve as potential indicators for non-communicable diseases, including oral cancer [5]. Because of its direct contact with oral tissues and easy, non-invasive collection process, saliva is particularly suited for oral cancer detection. Compared to blood, saliva is more convenient to collect—it does not clot and can be stored without specialized equipment. This has led to the emergence of the field known as "salivaomics," which explores the genomic,

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transcriptomic, proteomic, metabolomic, and RNA-related content of saliva for diagnostic purposes [6, 7].

Given its anatomical closeness to oral tumors, saliva serves as a promising medium for liquid biopsy—a technique involving the analysis of various body fluids for the presence of tumor-derived components. These may include exosomes, platelets, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and cell-free RNA (cfRNA) [8, 9].

Liquid biopsy enables real-time monitoring of tumor biology, offering insights into tumor heterogeneity, disease progression, residual disease, and metastatic burden. It provides a dynamic molecular snapshot of the tumor, enhancing early detection and personalized treatment approaches. Biomarkers such as CTCs, exosomes, ctDNA, and miRNAs contribute significantly to the diagnostic power of this method. This review presents a comprehensive discussion of the current landscape of these molecular markers—ctDNA, CTCs, and exosomal miRNAs—in the context of oral cancer detection, clinical management, and surveillance.

Materials and Methods

To perform a thorough review of the literature on the role of liquid biopsy in diagnosing oral squamous cell carcinoma (OSCC) and identifying associated biomarkers for cancer detection and therapy, an extensive database search was carried out. Databases consulted included PubMed, Web of Science, EMBASE, Scopus, and Google Scholar. The search strategy employed a combination of keywords, including: ((liquid biopsy OR oral biopsy)) AND (oral cancer, head and neck cancer, oral squamous cell carcinoma, OSCC, mouth cancer) AND ((saliva OR salivomics OR prognostic biomarkers)) AND ((oral cancer detection OR biomarkers OR diagnosis and treatment)).

Duplicate entries and non-English publications were excluded. The titles and abstracts of 431 studies were initially reviewed, with ineligible or unrelated studies being removed. Additionally, papers without accessible full-text versions were excluded. Following this screening process, 35 studies were selected for inclusion, all of which met the predetermined criteria. These studies addressed diverse aspects of liquid biopsy in OSCC, ranging from the identification of novel biomarkers to the development of diagnostic techniques, with several focusing specifically on saliva-based diagnostic approaches.

Results and Discussion

The reviewed literature emphasizes the significant promise of liquid biopsy as a non-invasive method for diagnosing oral cancer. It also highlights the emerging role of novel biomarkers in enhancing the accuracy and efficiency of cancer detection and treatment strategies. Despite the encouraging findings, further investigation is necessary to validate and expand the clinical utility of liquid biopsy technologies and biomarker-based assessments in the context of oral cancer.

Circulating tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) consists of fragmented genetic material shed by cancer cells, which can be detected in blood and saliva. In patients with OSCC, ctDNA levels offer valuable diagnostic and prognostic information, as they tend to rise with disease progression and typically remain in bodily fluids for less than two hours. This rapid turnover enables real-time monitoring of tumor dynamics throughout treatment [9–11].

Moreover, ctDNA analysis allows for the detection of somatic mutations in genes such as *TP53*, *CDKN2A*, *NRAS*, *NOTCH1*, *PIK3CA*, *HRAS*, and human papillomavirus (HPV) strains 16 and 18. Additionally, hypermethylation patterns in genes like *EDNRB*, *KIF1A*, and *HOXA9* found in salivary DNA serve as early indicators of OSCC, providing a basis for early detection and diagnostic accuracy [12].

Exosomes

Exosomes are nano-sized extracellular vesicles, typically ranging from 40 to 160 nanometers in diameter, actively secreted by nearly all cell types. Their formation begins with the inward budding of the plasma membrane, resulting in the creation of multivesicular bodies (MVBs). These MVBs can interact with various intracellular structures, including the Golgi apparatus, endoplasmic reticulum, and other vesicular systems [13, 14]. Depending on the cellular environment, origin, and metabolic activity, MVBs may fuse with lysosomes, autophagosomes, or the plasma membrane to release exosomes [15].

Given their abundance in bodily fluids, exosomes are considered a promising component in liquid biopsy applications [16, 17]. In fact, among all analytes used in this approach, exosomes are the most prevalent—concentrations can reach up to 10^{11} particles per milliliter of blood. In cancer patients, tumor-derived exosomes

constitute up to 10% of the total exosomal population. These vesicles mirror the molecular contents of their parent cells, including DNA, RNA, lipids, metabolites, and surface proteins [15].

Unlike other analytes in liquid biopsy, exosomes have a distinct advantage: they contain a rich variety of RNA types and preserve both RNA and DNA that reflect tumor-specific mutations [18]. Notably, exosomal DNA can carry the entire genome and mutational landscape of the originating tumor, whereas ctDNA is typically fragmented and less comprehensive [19]. This makes exosomes a particularly powerful tool for detecting and analyzing cancer-specific biomarkers, either independently or in conjunction with other markers. Additionally, fluctuations in exosome release may serve as a clinical metric to assess disease progression or treatment response.

Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are malignant cells that have detached from either a primary tumor or metastatic site and entered the bloodstream. They share many genetic mutations with the original tumor cells and may travel individually or in cell clusters, often indicating a greater likelihood of metastasis. To disseminate through the bloodstream and initiate secondary tumor growth, CTCs must undergo a complex multistep process known as the "metastatic cascade." These cells are exceptionally rare—only about one CTC is found per 10 million blood cells in patients with metastatic disease.

CTCs are pivotal in the context of personalized oncology. Their detection could provide a powerful means for predicting the emergence of metastatic disease in oral cancer, potentially enabling timely therapeutic interventions aimed at controlling or preventing disease progression. As one of the primary targets in liquid biopsy, CTCs can be enriched and identified using a range of technologies, offering an accessible and minimally invasive source for cancer profiling and monitoring. They carry key information related to tumor biology, including invasiveness, therapeutic response, and resistance mechanisms.

CTCs play a critical role in the spread of cancer. Current evidence suggests that their elimination could play a role in halting metastasis. Since they act as precursors to metastatic tumors, targeting CTCs may help reduce their circulation in the bloodstream, prevent secondary tumor formation, and ultimately lower the tumor burden in cancer patients [18].

Furthermore, CTCs serve as valuable biomarkers for early cancer detection [20, 21]. Molecular and cellular analysis of these cells can inform prognosis and support clinical decision-making [22, 23]. Given that tumor recurrence and metastasis are hallmarks of cancer, CTCs also represent a reliable indicator for evaluating tumor aggressiveness and guiding therapy selection. Continued exploration of their biological features may enhance their application in dynamic patient monitoring and treatment planning.

The emerging role of MicroRNAs (miRNAs) as liquid biopsy biomarkers for early cancer diagnosis

MicroRNAs (miRNAs) are small, non-coding RNA molecules—approximately 25 nucleotides in length—that are crucial regulators of gene expression. Over the past decade, miRNAs have gained considerable interest as diagnostic indicators for various malignancies, including oral cancers. Their stability in bodily fluids is largely attributed to their association with Argonaute 2 (Ago2) protein complexes, high-density lipoproteins, or their encapsulation within extracellular vesicles (EVs). These features enable their detection in fluids such as blood, saliva, and urine using advanced platforms like next-generation sequencing, microarray technology, and conventional PCR techniques [24, 25].

Recent investigations have emphasized the potential of miRNAs in noninvasive cancer detection strategies. For example, a large-scale Japanese study developed a machine-learning model capable of predicting cancer types using miRNAome data, achieving an overall accuracy of 0.88, which improved to 0.90 for early-stage cancers [26]. In another study, Nakamura *et al.* [27] demonstrated that distinct serum miRNA expression profiles could effectively distinguish oral cancer patients from healthy individuals. Furthermore, Romani *et al*. identified a group of 25 miRNAs that were differentially expressed in OSCC patients compared to controls, while Mehterov *et al*. reported that a specific miRNA panel could diagnose OSCC with 98% sensitivity and 60% specificity [28, 29].

However, these findings stem from retrospective, case-control studies, leaving uncertainty about the timing of miRNA appearance in biological fluids during disease progression. To validate their utility in clinical practice, future research should focus on large-scale, prospective cohort studies that explore the temporal dynamics, diagnostic accuracy, and clinical relevance of miRNA-based biomarkers. If confirmed, the integration of

miRNA profiling into routine screening could greatly enhance early cancer detection and treatment outcomes.

Cell-Free DNA (cfDNA) and its diagnostic relevance in oral squamous cell carcinoma

Cell-free DNA (cfDNA) refers to fragmented DNA released into the bloodstream and other body fluids as a result of cellular apoptosis or necrosis. In cancer diagnostics, cfDNA derived from tumor cells—commonly referred to as circulating tumor DNA (ctDNA)—is characterized by specific somatic mutations that distinguish it from normal cfDNA [30]. These DNA fragments are present in fluids such as blood, plasma, saliva, urine, and cerebrospinal fluid, providing a noninvasive avenue for genetic analysis [31].

In the context of oral squamous cell carcinoma (OSCC), cfDNA has recently been investigated for its potential diagnostic applications. Although some studies have not observed significant differences across patient groups, others have identified promising trends. Perdomo *et al.* [32], for instance, analyzed TP53 mutations in head and neck cancer patients by comparing ctDNA sequences from plasma, tumor tissues, and oral rinses, showing the feasibility of mutation detection across multiple sample types.

Moreover, detection of human papillomavirus (HPV) using cfDNA has shown promise in HPV-associated OSCCs. Mazurek *et al.* [33] demonstrated that cfDNA testing could aid in the early identification and ongoing monitoring of HPV-positive head and neck cancers. In their study, 14% of patients tested positive for HPV, with HPV16 accounting for 96.4% of cases. Other studies have evaluated methylation markers such as SPEPT9 and SPEPT3 in biofluids, which have shown high diagnostic accuracy, particularly in cancers beyond the oral cavity.

Despite these advances, several obstacles remain. Challenges include detecting ctDNA at low concentrations in early-stage tumors, accounting for tumor heterogeneity and clonal evolution, and developing standardized, multiplexed assays for broad clinical use **(Figure 1)**. Addressing these issues will be essential to fully harness the diagnostic and prognostic value of cfDNA analysis in OSCC.

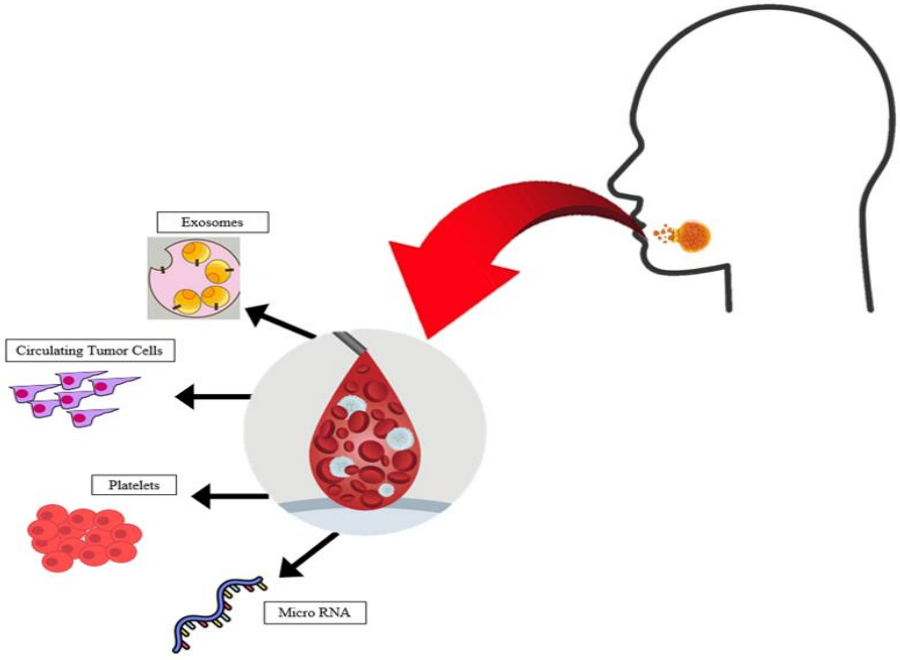

Figure 1. The essence of using saliva as a liquid biopsy in oral cancer diagnostics.

Future directions

Liquid biopsy represents a promising, noninvasive approach for early cancer detection, molecular characterization, tracking treatment responses, and identifying minimal residual disease. Despite its broad potential, its current application in oral cancer remains limited and calls for further comprehensive studies. The advent of personalized medicine has accelerated efforts to create a robust panel of highly specific and sensitive circulating biomarkers aimed at enhancing diagnostic

accuracy and prognostic predictions in patients with oral malignancies [34].

Emerging research has demonstrated that liquid biopsy can identify oral cancer-specific molecular alterations in circulating tumor-derived DNA, RNA, and proteins [2]. These molecular signatures may serve as valuable tools for early diagnosis, monitoring therapeutic responses, and assessing disease prognosis [3]. Moreover, liquid biopsy offers insights into the clonal evolution of oral tumors, thereby unveiling novel therapeutic targets [4]. Understanding the biology and origin of these circulating biomarkers—particularly tumor-derived exosomes (TDEs), which carry RNA, proteins, and other molecules—can significantly refine the precision of liquid biopsy technologies [6].

Incorporating a well-defined biomarker panel could serve as an effective substitute for invasive tissue biopsies, facilitating more accessible, cost-effective surveillance of disease progression and response to treatment. However, as the application of liquid biopsy in oral cancer remains in its developmental phase, expansive multicenter, prospective clinical trials are essential to validate its diagnostic and prognostic potential. Such investigations could ultimately drive advancements in targeted therapies and personalized care strategies for individuals diagnosed with oral cancer.

Conclusion

Liquid biopsy presents a reproducible and minimally invasive diagnostic approach that delivers real-time, cancer-specific molecular data. It has demonstrated considerable promise in the detection and monitoring of oral cancer, offering distinct advantages over traditional histopathological methods. Although key molecular features—including the proteome, metabolome, microRNAome, extracellular vesicles, circulating tumor DNA, and tumor cells—have not yet been fully established as reliable biomarkers for oral cancer, they hold considerable potential for future diagnostic use.

To translate this potential into clinical practice, further research is needed to validate these biomarkers and confirm their utility in guiding oral cancer treatment. With ongoing innovation and comprehensive clinical evaluation, liquid biopsy could revolutionize how oral cancer is diagnosed and managed in the era of precision medicine.

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