Review Article

Salivary Circulating Tumor Cells as a Transformative Tool in Noninvasive Cancer Diagnostics: A Review

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Abstract

Oral squamous cell carcinoma remains a global health challenge due to its notable morbidity and mortality rates. Early detection and effective monitoring are critical for improving patient outcomes, as early-stage diagnosis is strongly correlated with better prognosis. However, conventional diagnostic methods such as tissue biopsies and blood tests are invasive, limiting their utility for regular monitoring. Recent advancements highlight the potential of detecting tumor cells in saliva as a noninvasive, accessible, and cost-effective alternative. Saliva-based tumor cell detection offers a particularly promising avenue for diagnosing and monitoring oral and oropharyngeal cancers. This review explores the feasibility, advantages, and challenges of utilizing saliva-based tumor cell detection for early diagnosis, disease monitoring, and personalized treatment strategies.

Keywords
- salivary tumor cells
- oral squamous cell carcinoma
- noninvasive diagnostics
- early detection
- liquid biopsy

Introduction

Oral squamous cell carcinoma (OSCC) accounts for a notable global health burden, with a 5-year survival rate remaining at approximately 50% despite advancements in diagnosis and treatment.[1] The primary factors contributing to poor outcomes are late-stage diagnosis, metastasis, and recurrent disease. Effective diagnostic tools that facilitate early detection and consistent disease monitoring are critical for improving prognosis. However, traditional diagnostic approaches, including biopsies and blood-based tests, are invasive, expensive, and unsuitable for frequent use.[2] In this context, saliva offers a novel, noninvasive medium for cancer diagnostics.[3] Saliva's accessibility, ease of collection, and continuous interaction with the oral cavity make it an attractive alternative for identifying biomarkers associated with oral cancers.[4] Among these biomarkers, tumor cells shed into saliva, referred to here as salivary tumor cells (STCs), represent a transformative tool in the diagnosis and monitoring of OSCC. This review examines the clinical potential of STCs, their detection methodologies, and the associated challenges in harnessing this technology for routine cancer diagnostics. While numerous studies have extensively investigated circulating tumor cells (CTCs) in peripheral blood across various cancers, there is a notable lack of published data specifically addressing the presence and diagnostic utility of tumor cells in saliva. Therefore, this review draws upon the foundational understanding of CTC biology and detection technologies as a reference point to explore the potential of detecting tumor-derived cells in saliva. We propose the term STCs to differentiate these locally shed cells from blood-borne CTCs, emphasizing their unique biological context within the oral cavity. This conceptual framework aims to stimulate further clinical and translational research in this emerging domain.

Revisiting Terminology: Why STCs?

CTC is commonly used to describe cancer cells that enter the bloodstream and circulate through the body.[5] However, in the context of saliva, these cells are not "circulating" but are

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primarily exfoliated or shed from primary tumors in the oral cavity or surrounding regions.[6] Several mechanisms facilitate the entry of tumor cells into saliva. One route is direct shedding, where tumor cells are exfoliated directly from the primary site into the saliva.[7] Another is through exosomal release, wherein tumor-derived exosomes carrying specific genetic or phenotypic markers are secreted into saliva.[8] Passive diffusion is also possible, allowing tumor cells to enter saliva as a result of necrosis or other passive mechanisms. Additionally, in rare instances, translocation may occur, where tumor cells migrate from the systemic circulation to the salivary glands before appearing in saliva.[9]

To reflect this distinction and avoid confusion, alternative terminologies are proposed:

- Tumor cells in saliva—: Directly describes their source without implying circulation
- STCs: Captures tumor-derived biomarkers, including cells, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins
- Detached or exfoliated tumor cells: Highlights their detachment from tumor masses

This review adopts the term STCs to encompass all tumor-derived cells detectable in saliva.

STC Biology

STCs are likely to originate directly from primary oral lesions through mechanisms such as passive exfoliation, active detachment, or alterations within the tumor microenvironment.[10,11] Their shedding is influenced by factors like epithelial disruption from invasive growth, secretion of proteolytic enzymes (e.g., matrix metalloproteinases) that weaken cell–matrix adhesion, the induction of epithelial–mesenchymal transition (EMT) facilitating motility and resistance to cell death, and chronic inflammation that alters epithelial turnover.[12–14] STCs are more likely to be released into saliva from ulcerated or exophytic lesions, particularly in areas subject to mechanical friction or trauma. Once in saliva, tumor cells face a hostile environment characterized by enzymatic degradation, antimicrobial components such as secretory immunoglobulin A, fluctuating pH, and constant interaction with the oral microbiota.[15,16] To survive these conditions, STCs may remain embedded in mucus, be encapsulated within exosomes or microvesicles, and exhibit phenotypic adaptability —such as expressing mucin-associated or EMT-related proteins—that enhances their resilience.[17,18]

CTC Survival Mechanisms: Insights for STCs

Tumor cells to survive in harsh environments like the bloodstream exhibit the following adaptive mechanisms: *Immune evasion* that is facilitated by the expression of proteins such as HER2 and PD-L1, which help tumor cells escape immune detection and contribute to chemoresistance.[19] Additionally, *platelet shielding* occurs when tumor cells form aggregates with platelets, providing protection from immune cells and shear forces.[20] *Genetic plasticity* further supports tumor survival, as the high genetic diversity of tumor cells contributes to both treatment resistance and increased metastatic potential.[21] These insights, though derived from studies on CTCs in blood, underline the importance of targeted approaches to STC detection and analysis in saliva. ►**Tables 1** and **2** provide a

Table 1 Comparison of CTCs and STCs

Feature	Circulating tumor cells (CTCs)	Salivary tumor cells (STCs)
Origin	Intravasation from primary/metastatic tumor into blood	Shedding from oral mucosal tumors directly into saliva
Microenvironment	Hemodynamically active, immune surveillance	Enzyme-rich, variable pH, mucosal immunity, microbiota -rich
Survival mechanisms	EMT, immune evasion	Mucus embedding, exosomal transport, EMT features
Metastatic role	Systemic dissemination and metastasis	Local invasion marker, possible lymphatic spread
Accessibility	Requires venipuncture, preprocessing	Noninvasive, simple saliva collection
Diagnostic utility	Established in many cancers (e.g., breast, lung, prostate)	Emerging concept in oral cancers
Challenges	CTCs are extremely rare, often fewer than 10 cells/mL of blood amidst millions of normal blood cells. This low abundance requires ultrasensitive detection technologies, increasing cost and complexity CTCs undergo mechanical and oxidative stress while circulating, making them fragile and susceptible to lysis or loss of viability during collection and processing[22] CTC detection is often biased toward epithelial markers (e.g., EpCAM). Tumor cells undergoing epithelial–mesenchymal transition (EMT) may downregulate these markers, leading to false negatives[23]	High degradation risk, epithelial contamination which reduces specificity and makes it difficult to distinguish malignant cells from benign ones. Saliva's microbes and mucins can interfere with staining and molecular tests, leading to unclear or false results[24]

Abbreviations: EpCAM, epithelial cell adhesion molecule; EMT, epithelial–mesenchymal transition.

Table 2 Comparative analysis STCs vs. serum-based CTCs

Variable	Serum-based CTCs	STCs
Collection	Venipuncture (invasive)	Saliva sample (noninvasive)
Concentration	Higher in systemic cancers	Lower; localized to oral cancers
Clinical insights	Systemic tumor spread	Local tumor progression
Ease of access	Moderate	High
Cost	Higher	Lower

Abbreviations: CTC, circulating tumor cell; STC, salivary tumor cell.

comparative summary of CTC versus STC and serum-based CTC versus STC, respectively.

Clinical Applications of STC Detection

Saliva-based diagnostics hold immense potential for noninvasive cancer care. Some of the applications include: They can serve as biomarkers for early-stage OSCC, potentially reducing diagnostic delays. STCs also enable frequent monitoring of tumor progression and response to therapy. Additionally, molecular profiling of STCs supports personalized treatment strategies tailored to individual patients. Moreover, saliva collection proves particularly valuable for screening in high-risk populations, especially in low-resource settings or for large-scale cancer screening programs.

Isolation Techniques

Efficient isolation of STCs is critical due to their low abundance in saliva compared to other cellular components. Several advanced methods have been developed to enhance specificity and sensitivity:

1. Filtration:
   Filtration-based systems operate on the principle of separating cells based on size. Tumor cells, being larger than most other cells and debris in saliva, are retained while smaller components pass through the filter. The technology involves the use of specialized membrane filters with defined pore sizes (e.g., 8–10 µm) to effectively capture tumor cells.
   - Advantages:
     ✓ Simple and cost-effective
     ✓ Suitable for large-scale studies
   - Limitations:
     ✓ May capture nontumor large cells, reducing specificity
     ✓ Potential for clogging during processing

2. Immunomagnetic separation:
   - *Immunomagnetic separation* is based on the principle of using magnetic beads coated with antibodies specific to epithelial or tumor markers such as EpCAM (epithelial cell adhesion molecule). These beads selectively bind to tumor cells, which can then be isolated magnetically.[25] The technology employs advanced bead designs and antibody conjugation to ensure high affinity and specificity. After separation, the captured cells can be released for downstream analysis using gentle enzymatic or chemical treatments.
   - Advantages:
     ✓ High specificity for tumor cells
     ✓ Can isolate live cells suitable for further culture or analysis
   - Limitations:
     ✓ Dependency on marker expression, can vary across tumor types
     ✓ Expensive due to use of specialized reagents

3. Microfluidic devices:
   - *Lab-on-chip devices* operate on the principle of leveraging the physical and biochemical properties of tumor cells for automated isolation. These devices utilize microscale channels and chambers to selectively capture cells based on size, deformability, or surface markers. The technology includes integrated systems with optical sensors for real-time monitoring, and advanced designs that allow for parallel processing of multiple samples.
   - Advantages:
     ✓ Highly precise and automated, reducing human error
     ✓ Capable of handling small sample volumes
   - Limitations:
     ✓ High initial cost for device fabrication
     ✓ Requires technical expertise for operation and maintenance

Characterization Approaches

Once isolated, STCs undergo detailed characterization to confirm their malignancy and to derive clinically relevant insights. These approaches utilize cutting-edge imaging and molecular biology techniques:

1. Morphological analysis:
   - *Methodology*: Tumor cells exhibit unique morphological features such as: enlarged nuclei with irregular shapes, high nuclear-to-cytoplasmic ratio, prominent nucleoli, and mitotic figures, indicating active cell division[26]
   - *Tools required*: Light microscopy, phase-contrast microscopy, or fluorescence microscopy with specific

dyes to enhance visualization and imaging software for automated analysis of cellular features
- *Clinical utility*: Provides quick confirmation of malignancy based on visual criteria
- *Limitations*: Morphological overlap with nontumor cells can lead to false positives, requiring additional molecular confirmation

2. Molecular profiling:
   - Techniques:
     ✓ *Reverse transcription-polymerase chain reaction*: Amplifies specific tumor-associated messenger RNA or DNA markers, such as mutations in TP53 or human papillomavirus DNA
     ✓ *Fluorescence in situ hybridization*: Detects chromosomal abnormalities and specific genetic alterations (e.g., amplifications or deletions) in individual cells[27]
   - Advantages:
     ✓ High sensitivity and specificity in detecting genetic and epigenetic alterations
     ✓ Ability to identify actionable targets for personalized therapy
   - Applications:
     ✓ Differentiating between malignant and nonmalignant cells
     ✓ Monitoring treatment response through changes in molecular profiles

3. Flow cytometry:
   - *Principle*: Tumor cells are labeled with fluorescent antibodies targeting tumor- specific markers (e.g., EpCAM, cytokeratins, or PD-L1). As the cells pass through a laser beam, fluorescence intensity is measured to identify and quantify the tumor cells[28]
   - Technology:
     ✓ Multicolor flow cytometry enables simultaneous detection of multiple markers
     ✓ Sorting capabilities allow isolation of specific subpopulations for further study
   - Advantages:
     ✓ Rapid and high throughput
     ✓ Quantitative, providing precise cell counts
     ✓ Can distinguish between live and dead cells using viability dyes
   - Limitations:
     ✓ Requires sophisticated equipment and trained personnel
     ✓ High reagent costs

Emerging Technologies in STC Detection

In addition to the above methods, ongoing advancements aim to overcome existing challenges. *Single-cell sequencing* provides unparalleled insights into the genetic and epigenetic landscape of individual tumor cells, aiding in the identification of novel biomarkers and therapeutic targets.[29] *Artificial intelligence (AI)-based image analysis* allows the integration of AI in microscopy and imaging, enabling automated, accurate classification of tumor cells and reducing observer bias. *Nanotechnology* is also being employed, with nanoparticle-based systems developed to enhance the sensitivity of detection techniques, particularly for rare tumor cells in saliva.[30] These innovations promise to make STC detection more robust, accessible, and clinically impactful, paving the way for their integration into routine cancer diagnostic.

Challenges

Detecting and processing STCs present multiple technical and biological challenges. The extremely low concentration of tumor cells in saliva, especially in early-stage cancers, makes detection difficult, while their heterogeneous nature complicates the development of standardized identification protocols. Additionally, the high risk of microbial contamination and the presence of nontumor components such as epithelial cells and debris in saliva can pose significant challenges for culturing and accurate analysis, requiring the use of antimicrobial agents. The fragile nature of tumor cells and their biomarkers in saliva can affect the stability and reliability of analysis, while the lack of validated, sensitive, and specific detection tools continues to limit clinical applicability. Overall, significant standardization and technological advancement are needed before saliva-based tumor cell diagnostics can be reliably integrated into routine clinical practice.

Strengths

STCs have key advantages over CTCs, especially for oral cancer. STCs can be collected noninvasively through saliva, improving patient comfort and compliance. They are cost-effective, easy to repeat, and suitable for large-scale screening. Unlike CTCs, STCs reflect local tumor activity, allowing earlier detection of oral lesions. Their potential for real-time, localized monitoring positions STCs as a promising tool in oral cancer diagnostics, despite the need for further validation and standardization.

Gray Areas

STCs face limitations due to the lack of standardized detection methods and variability in saliva composition. Low cell yield, contamination, and degradation make isolation difficult. STC diagnostics are still experimental, with limited validation and accuracy compared to CTCs, requiring further research.

Generalizability

The generalizability of STCs as diagnostic markers remains limited. Without broader validation across diverse populations and cancer types, STCs cannot yet be reliably applied as a universal diagnostic tool.

Conclusion

STCs represent a paradigm shift in noninvasive cancer diagnostics, offering a simple and patient-friendly alternative for detecting and monitoring oral cancers. Despite challenges such as low abundance and heterogeneity, ongoing advancements in detection technologies and molecular analysis are steadily enhancing their feasibility and reliability. By addressing current limitations through innovation and validation studies, saliva-based tumor cell diagnostics could transform cancer care, making it more accessible, personalized, and effective.

Authors' Contributions

All authors S.S.R., A.R., and A.A. have equally contributed to manuscript writing and data collection.

Patient Consent

Patient consent is not required.

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Conflict of Interest

None declared.

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