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Original Research Article

The Potential of Circulating Tumor Cells as Biomarkers for Oral Cancer Detection and Monitoring

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Abstract:

Circulating tumour cells (CTCs) are cancer cells that have detached from the tumour and are now floating around in the blood. Their ability to serve as biomarkers in the detection and tracking of cancer has been acknowledged. A less invasive diagnostic approach called a liquid biopsy may be utilized to discover CTCs in blood. By identifying circulating biomarkers such as exosomes, circulating tumour DNA (ctDNA), proteins, and microRNAs (miRNAs), liquid biopsy can enhance oral cancer diagnostic procedures and pave the way for tailored therapy. The late diagnosis of oral cancer, which increased morbidity and death rates, remained a significant health problem worldwide. While conventional diagnostic procedures did a good job overall, they only sometimes had the specificity and sensitivity needed for monitoring and early detection. Oral malignancies were among the many tumours for which circulating tumour cells (CTCs) had shown promise as biomarkers. The possibility of CTCs as non-invasive indicators for the identification and monitoring of oral cancer was discussed in that abstract. It also emphasized new technologies that had recently emerged, such as nanotechnology and microfluidics that helped isolate and analyze CTCs. Additionally, that presentation explored clinical research that showed how CTCs might have been helpful for oral cancer patient's prognosis, therapy response evaluation, and monitoring. The problems and limitations of CTC-based tests were also discussed, highlighting the need for more extensive validation studies and standardized methodologies. The use of CTCs as biomarkers had the potential to significantly improve patient outcomes in the management of oral cancer via the implementation of tailored treatment plans, early identification, and real-time monitoring. Due to the high cost of the technologies used to detect CTCs and the tiny numbers seen in blood, a common standard has yet to be established. Additional research is necessary to determine the DNA types of all cancers and to develop methods that detect CTCs with higher sensitivity and precision.

Keywords: CTC, DNA, Biomarkers, Oral Cancer, Tumor.

Introduction

The mouth and throat are potential sites for the development of oral cancer. It is one of the most frequent cancers worldwide, with over 350,000 new cases reported yearly and 177,000 fatalities. [1] Squamous cell carcinomas (OSCCs) originate on the surface epithelium and account for about 90% of oral malignancies. [1] The number of OSCC cases has gone down in certain areas but up in others, especially in countries with low incomes and among women. [2] Among those under the age of 45, there has been an alarming upsurge in the incidence of OSCC. [1]

Oral cancer is infamously challenging to detect due to the lack of symptoms and the difficulty in differentiating between benign and malignant tumours. However, circulating tumour cells (CTCs) have recently been explored as a potential biomarker for oral cancer detection and tracking. Central tumour cells (CTCs) are cancer cells that have spread to the bloodstream and are no longer part of the primary tumour. [3] If physicians detect these cells in the blood, it might provide valuable information on the presence and progression of the malignancy. Many challenges arise while attempting to isolate, detach, and identify CTCs because of characteristics, including their heterogeneity and rarity. [4]

With over a hundred indicators demonstrating variable levels in individuals with oral squamous cell carcinoma (OSCC), saliva is a biofluid that has great diagnostic promise in this disease. Molecular components of cell surfaces, pieces of the cytoskeleton, proteins inside cells, proteases, and proteins linked to inflammation, signatures of messenger RNA, and noncoding RNA are all examples of biomarkers. The molecular features

and severity of OSCC are reflected in the high sensitivity and specificity of sure of these biomarkers.

Inorganic ion concentration, telomerase level, specific oral microbiota, metabolic and oxidative stress indicators, and salivary-mutated and salivary-methylated DNA are among the other biomarkers. Stable detection and straightforward quantification are made possible by the stabilization of the RNA in exosomes. In multiethnic cohorts of oral cancer patients, the salivary transcriptome, which includes coding and noncoding RNAs, has shown efficacy. In general, biomarkers provide hope for bettering oral cancer detection, diagnosis, and prognosis via early insight into the disease's dynamics. [5]

Improving patient prognosis and survival rates requires oral cancer identification and surveillance. A new and intriguing possibility for this function is circulating tumour cells (CTCs), which have broken out from the primary tumour and are now floating around in circulation. Their identification may provide essential details on the existence and advancement of cancer. However, there are obstacles, such as CTC identification, isolation, and detachment, not to mention the rarity and variability of CTCs. [6]

It is thrilling to think of CTCs' potential as biomarkers for monitoring and detecting oral cancer, which might lead to better diagnosis and therapy. Early identification is sometimes tricky owing to the absence of particular symptoms and the difficulties in differentiating between benign and malignant tumours; detection in the blood might facilitate this process. Information on the efficacy of treatments and the advancement of diseases might be gleaned by monitoring CTCs as well. Nevertheless, in order to overcome these obstacles and prove their practical use in the diagnosis and monitoring of oral cancer, more study is required. [7]

Biology of CTCs

The term "circulating tumour cells" (CTCs) describes cancer cells that have extravasated into the circulation after separating from the primary tumour. Metastases, which are secondary tumours that form as cancer cells migrate from the primary tumour to other areas of the body, are caused by them.

Degradation of the extracellular matrix and activation of enzymes are involved in the complicated process of CTC release. Cancer stem cells (CTCs) diverge significantly from the original tumour in both genetic and phenotypic ways, making them very diverse. Various factors, including immune surveillance and shear stress, act as selective forces as they move through the circulation, resulting in this variability. CTCs have been investigated as potential biomarkers for cancer diagnosis and surveillance because of the rich information they may give on the existence and course of disease. [8]

These cells, which detach from primary tumours and circulate throughout the body, have shown diagnostic and prognostic use. The development of new high-throughput technologies has allowed for the extraction of these cells from blood, which has paved the way for single-cell research and offered a precise, dynamic, and treatment-related approach to cancer therapy in both sickness and treatment. [9]

Molecular Biomarkers

Recent years have seen a surge in the therapeutic significance of circulating tumour biomarkers as a tool for early cancer identification and therapy monitoring.

These indicators provide light on tumours' microenvironments and genetic and epigenetic changes, which may help in the search for new biomarkers and treatment targets in the age of precision medicine. Potentially enhanced cancer clinical evaluation and treatment approaches might result from analysis of ctDNA levels, DNA methylation, and point mutations. One potential approach for the diagnosis and prognosis of mouth cancer and other malignancies is the detection of changed miRNA levels in bodily fluids such as blood and saliva. [13]

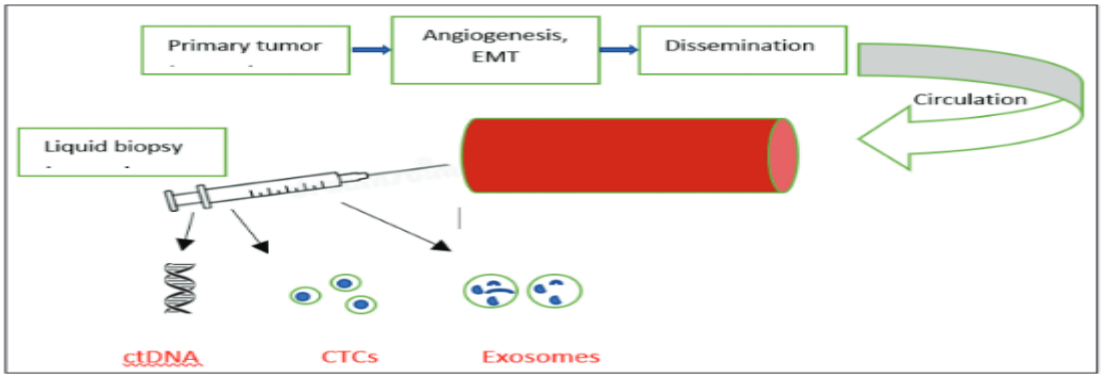

Figure 1: Visual depiction of biomarkers in circulation. [12]

Research Methodology

In order to determine if circulating tumour cells (CTCs) might be beneficial as biomarkers for the early diagnosis and monitoring of oral cancer, a prospective observational study called "The Potential of Circulating Tumor Cells as Biomarkers for Oral Cancer Detection and Monitoring" is now underway and study aimed to evaluate the efficacy of CTCs as biomarkers for the identification and surveillance of oral cancer. Prioritizing the comfort and ethical issues of participants, the research follows those who have been diagnosed with oral cancer. Patients' blood is taken both before and after treatment, with healthy controls' blood drawn as a reference point. Characterizing CTCs and identifying them are both done using fluorescence microscopy and particular markers. In molecular analysis, tools such as next-generation sequencing and polymerase chain reaction (PCR) are used to identify genetic mutations and molecular alterations. In order to gather and analyze data, it is necessary to measure and count CTCs, search for connections between CTC counts and clinical indicators, and monitor CTC dynamics before and after treatment by doing follow-up exams. Ensuring ethical considerations are met involves adhering to ethical standards and obtaining approval from relevant institutional review boards. This study intends to contribute to the existing body of knowledge by providing a comprehensive assessment of CTCs as potential biomarkers for the early detection and monitoring of oral cancer.

CTC Isolation Characterisation

When cancer cells break away from their original tumour and enter the circulation, they are known as circulating tumour cells (CTCs). It is not easy to isolate CTCs from peripheral blood; several approaches, including those based on density, size, and immunoaffinity, are involved in the process. Centrifugation, density-gradient centrifugation, fluorescence-activated cell sorting, magnetic-activated cell sorting, and buoyancy-activated cell sorting are some of the common approaches. [10]

These techniques separate different kinds of cells by targeting their unique characteristics, for example, RBCs, leukocytes, and cancer cells. Using fluorescently labelled antibodies, FACS identifies target cells, while antibody-coated magnetic beads adhere to specific cell surface markers. While BACS takes use of the buoyancy of cells in a density gradient, density-gradient centrifugation sorts cells according to their density using an angle of density medium. [11]

Results and Discussion

For early cancer identification and tumour heterogeneity monitoring, a less intrusive approach than traditional biopsy is a liquid biopsy. Clinical studies test CTCs and ctDNA, or circulating tumour cells, to determine their value for disease dynamics. Both approaches are particular and sensitive, but they each have their advantages when it comes to predicting outcomes and spotting relapses early on. Despite its FDA approval and use in clinical trials, the Menorini Cell Search TM system misses many CTCs and tiny clusters since it depends on the surface marker EpCAM for CTC enrichment. Parsortex and RareCyte are two such technologies that provide different approaches depending on cell density and size. [10]

In order to differentiate CTC from patients and healthy controls. When first detected, oral cancer is often asymptomatic or manifests with symptoms that are similar to those of less serious diseases. Like CTC, which is generally seen at late stages using imaging modalities like USG/CT/MRI, the lack of recognizable symptoms sometimes leads to delayed diagnosis. However, since there are no particular tumour biomarkers for oral cancer, the diagnosis is still primarily dependent on clinical and visual assessment.

Traditional tumour markers used for other types of cancer, such as CA125, CA19.9, CA242, CA15.3, CEA, and MDA, have shown inconsistent results and poor specificity when investigating oral cancer. This is in line with the difficulties encountered with diagnosing GBC, which further highlights the need for other techniques of oral cancer diagnosis.

Mandel and Métais discovered CfDNA in 1948, and it was shown that cancer patients had higher amounts of it compared to healthy controls. Despite its association with damaged cells in both benign and malignant illnesses, the exact mechanism for its circulation remains unclear. Another possible contributor is the DNA shedding by lysed CTCs into the circulation; nevertheless, the quantities of DNA in plasma and serum are higher than what low CTC counts can account for.

Investigating alternative indicators such as CTCs becomes more critical in the field of oral cancer diagnosis due to the absence of accurate and specific biomarkers. Oral cancer diagnostics continue to face obstacles similar to GBC; nevertheless, there is hope for better early detection tactics via the use of studies on CTCs as possible biomarkers.

Prospects for CTCs In The Treatment Of Oral Cancer

A potential technique for predicting disease progression and survival in metastatic and early-stage cancer patients is CTCs or cancer-specific tumour cells. These unique biomarkers may be identified at the cellular level and even cultivated or grown. Aggressive disease, more metastases, and shorter time to relapse are all associated with

high CTC counts. The presence of CTCs in individuals with oral cancer dramatically increases the likelihood of both local and distant metastases.

Their ability to accurately forecast when OSCC will recur makes them a valuable independent prognostic marker. Cancer tumour cells (CTCs) are involved in disease behaviour regulation; checkpoint inhibitors, which suppress the PD-1/PD-L1 immune checkpoint pathway on CTCs, encourage the immune system to eliminate CTCs from circulation, which may lessen the likelihood of disease recurrence and metastasis.

One way to track how well OSCC treatments are working is to look for signs of PD-L1 overexpression in CTCs. There is a noticeable difference in gene expression between primary tumours and CTCs, suggesting that CTCs may be shed from various parts of tumours and metastases.

Additional diagnostic and treatment options may be informed by expression profiling's ability to identify organ-specific metastatic signals. Due to the convenience and minimum invasiveness of sample collection, CTCs have the potential to function as a real-time marker for repeated evaluations of disease monitoring before, during, and after treatment.

Because they are non-invasive, reflect tumour mutational patterns, and may predict recurrence and local or distant dissemination, CTCs are excellent candidates for this role. [12]

Table 1: Applications CTCs

Cancer Type	CTCs Utility	Detection Methods	Molecular Characteristic	Main Findings/Purposes	Trial Identifier	Reference
Non-small cell lung cancer	Early detection, prognosis, and treatment monitoring	Size-based, immunoaffinity-based, and density-based methods	CTCs and ctDNA	The purpose of this research was to determine if CTCs and ctDNA are valuable indicators for detecting non-small cell lung cancer early on, predicting its prognosis, and tracking its response to therapy. 1.	NCT0451 1870	1
Breast cancer	Early detection, prognosis, and treatment monitoring	Size-based, immunoaffinity-based, and density-based methods	CTCs and ctDNA	To assess the clinical value of CTCs and ctDNA as breast cancer biomarkers for early identification, prognosis, and therapy monitoring. 2.	NCT0451 1870	2
Prostate cancer	Early detection, prognosis, and treatment monitoring	Size-based, immunoaffinity-based, and density-based methods	CTCs and ctDNA	The research assessed CTCs and ctDNA as prostate cancer indicators for early identification, prognosis, and therapy monitoring. 3.	NCT0451 1870	3
Colorectal cancer	Early detection, prognosis, and treatment monitoring	Size-based, immunoaffinity-based, and density-based methods	CTCs and ctDNA	The research assessed CTCs and ctDNA as biomarkers for colorectal cancer diagnosis, prognosis, and therapy monitoring. 4.	NCT0451 1870	4
Pancreatic cancer	Early detection, prognosis, and treatment monitoring	Size-based, immunoaffinity-based, and density-based methods	CTCs and ctDNA	The research examined the clinical value of CTCs and ctDNA as biomarkers for pancreatic cancer diagnosis, prognosis, and therapy monitoring. 5.	NCT0451 1870	5

Microfluidic Platforms

Because of their versatility, microfluidic platforms have changed the face of healthcare in many ways, including fertility testing, cell sorting, point-of-care diagnostics, infectious disease testing, DNA sequencing, tissue engineering, and biological and chemical analysis.

The functionalization of these micro devices with different antibody "cocktails" allows for more accurate extraction of CTCs, in addition to traditional anti-EpCAM proteins. Epithelial cell membrane antigens, EGFR, TROP-2, HER2, MUCI, human epidermal folate binding protein receptor, mesenchymal stem cell antigens, and growth factor receptor 2 are common biomarkers that are targeted in CTC isolation.

The CTC-Chip, made of micro-posts covered with antiEpCAM antibodies, is one of the micro devices that have been produced. The Herringbone (HB) CTC-Chip is an improved version of the original chip that has herringbone groove patterns. The redesign streamlines blood flow inside the channels, which improves capture efficiency by allowing more CTCs to come into touch with the antibodies that line the inside of the chip. A key tool for evaluating tumour metastasis, the HB-Chip captures CTC clusters and gives more detailed data.

With a self-assembling design that incorporates microfluidics and immunobead technology, a novel Ephesia CTC-Chip can quickly isolate and count CTCs with a capture efficiency of 90-94% and a non-specific capture rate of less than 0.4%. Problems arise, however, when trying to handle more significant sample quantities. Combining size-based enrichment with either EpCAM-based positive enrichment or CD45 negative depletion, the CTC iChip achieves a 97% capture yield and eight mL/h processing speeds, making it another practical example of separation technique combinations. In order to preserve cell viability, the device employs meagre flow rates in comparison to size-based filters.

A different method involves capturing CTCs according to their size, density, and compressibility using standing acoustic-wave fields inside microchannels. With a high input and the connection of a polydimethylsiloxane (PDMS) microfluidic channel to the substrate, this approach effectively separates CTCs with a recovery rate ranging from 83% to 90% while reducing the elimination of white blood cells (WBCs). Since cancer cells have lower densities than normal blood cells, the MagDense platform is designed to elevate cancer cells from the oesophagus, lungs, colon, and breast to different heights than normal blood cells. The selectivity and efficacy of this method for capturing CTCs from clinical samples need more research.

With an anti-EpCAM wire inserted directly into the peripheral arm vein, the GILUPI GmbH Cell Collector efficiently processes 1.5 L of blood in 30 minutes, capturing CTCs with exceptional efficiency. The detection rate of 22 out of 24 cancer patients (91.6% of the total) was achieved using CTC testing. The device's sensitivity is enhanced by its capacity to analyze such substantial blood volumes, making it a good choice for future CTC investigations. [4]

Detachment of CTCs

It is challenging to isolate cancer-specific cytokines (CTCs) from substrates using several methods without causing cell damage. The removal of receptor-ligand connections and focal adhesions is a necessary step in detachment procedures, although it may decrease cell survival. For cancer cells, less intrusive approaches have been developed, including temperature, light, electrodes, and aptamers. Three popular methods for CTC detachment technology—enzymatic digestion, aptamers, and pH-responsive polymers—are covered in this work. The goal of these techniques is to make these delicate cells more viable and enhance their function. [13]

Genomic Profiling of CTCs and Individualized Therapy

Many cancer treatments nowadays only target the tumour itself rather than its metastasized cells; as a result, patients may have a metastatic recurrence long after their initial diagnosis and surgical removal. In order to assess possible therapeutic targets and identify the underlying reason for aberrant functioning, molecular profiling of CTCs from patients with metastatic disease may be helpful. In order to better understand CTCs in relation to treatment choice and the development of customized medicine, a great deal of research has focused on genotypic and phenotypic characterization profiling.

Breast, prostate, and lung cancers are among the many tumour types that have undergone CTC characterization. A group studying breast cancer patients' gene profiles set out to examine CTCs for genetic variability and expression patterns. Gene analysis was conducted using the following cell lines: T47D, MCF7, MDA-MB-231, and SKBR3. The circulating tumour gene profiling study used the MagSweeper technology to collect cells and examine gene expression in 510 patients. Fifteen per cent of the cells (CTCs) studied regularly had a word of at least one of the 87 genes that were discussed. A complex disease model is derived from tumour cell molecular gene profiling. The seeding of metastases in patients is not the responsibility of all CTCs. There are only so many CTCs that can pull this off. Metastasis formation and aggressiveness are both increased in CTCs that

lack expression of ER, HER2, or PR. Because of this, the majority of treatments that aim to alter these biomarkers end up failing. [14]

The process of epithelial-mesenchymal transition (EMT), which involves the biological and morphological changes of epithelial cells to mesenchymal cells, is associated with genes such as ZEB2, TGFβ1, VIM, CXCR4, and FOXC1. This process increases cell invasion. Since pancreatic CTCs show WNT signalling in some human situations, molecular profiling of CTCs may serve as a potential target for therapy.

Drug studies may benefit significantly from CTCs. Twenty per cent of breast cancers in women are caused by HER2 amplification, which is also a biomarker for individuals with MBC. Typically, HER2-targeted medication is administered to patients whose HER2 status is over-expressed. Nevertheless, with further in-depth research on CTC characterization, we may get over the current lack of biomarker data and perhaps one day assess CTCs for both tailored treatments and molecular tissue screening. [15]

Conclusion

However, to determine their practicality in the clinic, more study is required. More effective approaches to diagnosing and monitoring oral cancer are needed, as the authors explain the limits of present diagnostic tools. Several methods for detecting CTCs are included in the paper as well. These methods include mass spectrometry, digital PCR, real-time PCR, and next-generation sequencing. There are still obstacles that restrict the present use of this growing diagnostic method, even though detection and molecular characterization have advanced. Researchers looked at people with oral cancer and discovered that CTC counts were correlated with clinical indications; this suggests that CTCs might be used to predict how well a patient would respond to therapy or how far along the illness will be in its course. Their use in real-time monitoring and recurrence identification is shown by their capacity to follow CTC changes during and after treatment. By systematically evaluating CTCs as biomarkers for early oral cancer diagnosis and surveillance, this study makes a substantial contribution to the current literature and opens the door to future investigations and possible therapeutic applications.

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