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## Circulating Tumor Cells: Novel Prognostic Indicators In Oral Squamous Cell Carcinoma

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### ABSTRACT

Oral squamous cell carcinoma (OSCC) is ranking 1<sup>st</sup> among males and 4<sup>th</sup> among females in India. The presence of circulating tumor cells (CTCs) in the blood vessels, acts as a significant indicator in developing metastases. It is important to identify patients suffering from non-localized tumor with “circulating” tumor cells. Malignant cells detached from the primary tumor are able to invade into under lying tissues, to intravasate into the bloodstream to become circulating tumor cells (CTCs), and becomes route cause for distant metastases. The early detection of metastases is an important indicator of survival, prognosis and relapse. Circulating Tumour Cells (CTCs) are exhibited in the peripheral blood of cancer patients. By utilizing certain tumor markers and by using multiple genetic markers, including cancer stem-like cells, we can assess the worth of the clinical importance of circulating tumor cells (CTCs) as a prognostic factor for overall survival (OS) and disease-free survival (DFS) in the peripheral blood (PB) of patients with cancer, who have undergone curative surgery.

**Keywords:** Oral squamous cell carcinoma, circulating tumor cells, prognostic factor

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## INTRODUCTION

Circulating tumor cells (CTCs) in the blood vasculature, play a significant role in laying a foundation for metastases. Metastasis causes death in 90% of cancer patients. Before the clinical symptoms arise, cancer cells are said to be detected in circulation [1]. Majority of sarcoma cases spread through metastasis initially with localized disease, and the most common site is the lungs [2]. With the world-wide incidence of oral squamous cell carcinoma reaching to around half a million persons, nearly 50% of patients die due to tumor-related complications. Early stage carcinomas are only seen in one third of the patients while two thirds present with advanced disease. More than 50% of patients suffering from head and neck squamous cell carcinoma (HNSCC) suffer local relapses while only up to 25% develop distant metastases. However, the prognosis of these patients remains poor [3].

Head and neck epidermoid carcinoma comprise tumors of the oral cavity, oropharynx, hypopharynx and larynx. The prognosis is mainly based upon the level of involvement of lymph nodes and metastasis. Although the treatment plan is improved, recurrence rate is 25 to 50% based on the stage and site. Salvage surgery is the only curative option for the recurrence case that is present above the clavicle [4]. Epidermoid carcinoma of the head and neck remains the sixth commonest of cancer-mortalities all over the world with estimates of around 650,000 new cases per year worldwide. Over the past 40 years, only 40–50% of patients with epidermoid carcinoma survive 5 years or longer with little variation to this number. The advanced treatment provides a better prognosis for local spread only but the metastatic disease remains a challenge [5]. For all stages of cancer the overall 5-year survival incorporated approximately 50% has not shown any sufficiently great changes for the past few decades [6]. The management of metastatic tumor remains a critical issue. The malignant cells dislodge from the primary cancer and obtain entry to circulation either directly through blood vessels or after transport in lymphatic channels. The liver, bone and lungs are the commonly involved secondary organs for the metastatic tumor [7].

Over the past few decades by the implementation of new surgical techniques and chemotherapy or bio-radiation protocol there is a significant raise in the localized control of squamous cell carcinoma of the head and neck region (SCCHN)<sup>4</sup>. Though the development in the clinical treatment significantly increased survival rates, the latter are still offset by a significant number of distant failures [8]. Using multiple genetic markers, including cancer stem-like cells, we can evaluate the clinical significance of circulating tumor cells (CTCs) as a prognostic factor for overall survival (OS) and disease-free survival (DFS) in the peripheral blood (PB) of patients with cancer, who have undergone curative surgery [9].

### DISTANT SPREAD VIA CIRCULATING TUMOUR CELLS

The detached tumor cells from invasive tumor front intravasate into the blood vessels in the form of clump or individual cells. Some cells are destroyed through immune surveillance and some escape as a result of skip metastasis. These cells in the blood vessel are represented as CTCs. Less than 2mm in the circulating blood is represented as micrometastases. Micrometastasis is very unusual in Head and Neck carcinoma [9].

### METHODS OF INVESTIGATION OF CIRCULATING TUMOUR CELLS

#### DETECTION OF CTCs BY FLOW CYTOMETRY

Flow cytometry is a laser- or impedance-based, advanced biophysical technology carried out in cell counting, cell sorting, biomarker detection and protein engineering, by suspending circulating tumor cells in a stream of fluid and passing them by an electronic detection apparatus. Flow cytometry is routinely used in the estimation of circulating tumor cells for diagnosis of cancers

#### DETECTION OF CIRCULATING EGFR TRANSCRIPTS BY NESTED RT- PCR

Cancer prognosis can be improved through early detection of CTCs by RT-PCR, since it produces unique mRNA transcripts based on the cancer type. Scientists are working on ways to use RT-PCR in cancer detection to help better prognosis, and monitor response to treatment. Circulating tumor cells develop unique mRNA transcripts based on the type of cancer. The prime goal is to determine which mRNA transcripts serve as

the best biomarkers for a particular malignant cell type and then evaluate its expression levels with RT-PCR [14].

#### **DETECTION OF P16INK4A THROUGH IMMUNOHISTOCHEMISTRY**

HPV POSITIVE + cases can be detected through the estimation of p16 expression in tumor tissue. Multiple samples are best demonstrated through tissue microarrays (TMAs). The hematoxylin and eosin staining technology is used as a common staining procedure.

#### **EPITHELIAL IMMUNOSPOTS**

The body covering is divided into two types-skin and mucous membrane. Skin represents the external outermost covering of the body which is directly exposed to outer environment and mucous membrane lines various cavities in the body associated with wet environment such as oral cavity, airways, intestinal tract, various internal organs and provide a significant interaction between the outside- environment and the body interior. Immune mechanism of the epithelium is regulated by leukocytes. Cytokeratin and cell surface receptors represent the immunospots [10].

#### **DETECTING CIRCULATING TUMOR MICROEMBOLI (CIRCULATING TUMOUR MICROEMBOLI DETECTION)**

The circulatory tumor micro emboli play a significant role in distant metastasis, which is emphasized in both clinical and animal studies. It is a collective of minimum two tumor cells and sometimes consists of normal blood cells. The existence of CTM depends upon the migration of tumor cells. Once the tumor cells are incorporated into the blood stream, there will be decreased expression of VEGF-A. The presence of CTM indicates higher metastatic potential than CTCs. Several methods have been employed to detect CTM, such as flow cytometry, Aptamer technology and sufficient expression with epcam and CKs [11].

#### **TECHNIQUES INVOLVED IN ISOLATION OF CTCs IN PATIENTS WITH RARE TUMORS**

In rare tumors, the isolation of CTC is done through the microfluidic system that consists of a microfluidic chip etched in silicon. A chip is enclosed by a manifold and the flow through the capture module is established by pneumatic pump. VEGF and FGF remains as specific markers for isolation of CTCs [12].

#### **DIFFERENTIATION BETWEEN CANCER STEM CELLS VS CIRCULATING TUMOR CELLS**

The most prominent and important feature of malignancy is its potentiality to proliferate and create new metastatic spots. In malignant condition CTCs play an important role in malignancies, as it remains the part of primary tumor which play a significant role in evolution of the disease and also responsible for metastases. Another important hallmark of cancerous disease is CSC. To isolate CTCs CD45, CD31, c-met, EPCAM, CD-227 and mmp-9 remains the standard markers. The undifferentiated embryonic stem cells are self-renewed by a transcription factor known as NANOG. The functions of NANOG is to match up with the OCT4 which forms the important criteria to identify the embryonic stem cells [13].

#### **CONCLUSION**

With various newer technologies available to detect circulating tumor cells, current staging methods of cancer have taken a new turn. Targeted therapy can be provided for patients suffering from oral squamous cell carcinoma. As detection of circulating tumor cells is non-invasive, patients can be easily motivated. As the five-year survival rates for oral squamous cell carcinoma remains low, detection of circulating tumor cells could suggest early metastasis and improve morbidity and mortality of patients through implementation of advanced therapeutic options.

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