REVIEW ARTICLE

# Tumor Microenvironment Tilling the Soil

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## ABSTRACT

The cells surrounding a tumor make up a molecular microenvironment known as the stroma. The stroma can be influenced and can in turn influence the growth and formation of tumors and new metastases. Origination of this microenvironment can be linked to the "seed and soil" concept with the original cancer cells, termed "seeds," and the microenvironment, termed "soil." The "soil" made up of the proteins, growth factors, and other non-tumor cells is a crucial part of tumor formation, invasion, and metastasis. It is important to consider and understand the interplay between the microenvironment and tumors when pursuing future therapeutics for cancers.

**Keywords:** Metastasis, Microenvironment, Molecular, Squamous cell carcinomas, Tumor.

## INTRODUCTION

Head-and-neck cancers are the sixth most common malignancy worldwide, and squamous cell carcinomas (SCC) comprise the majority of cases. SCC is the most common oral cavity cancer. It is the eight most common cancers in men and fifth most common in women. Tobacco use in various forms (smoking, chewing, and snuff dipping) and alcohol consumption both are major risk factors for oral cavity cancer. Frequent use beta-carotene and Vitamin E reduce the risk of oral SCC (OSCC).[1,2] Evidence shows that the human papillomavirus (HPV) has an oncogenic role; however, it is likely to be small. In spite 5 Years, survival rate after diagnosis remains low due to uncontrolled or recurrent tumors and lack of suitable markers for early detection.[3]

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## CLINICAL PARAMETERS

### Age and Sex

Age and sex were reportedly not associated with a survival rate in OSCC. According to Fan *et al*., the 5-year survival rate and disease-free survival rate were 61% and 75.5%, respectively, among OSCC patients under the age of 45.

### Tobacco and Alcohol

There is a higher survival rate in non-smokers and non-drinkers, but there is no difference between ever smokers and current smokers. Fang *et al*. showed that smoking was associated with an approximately 2-fold increase in the risk of recurrence and 5-fold increase in the risk for disease-related death.

### Tumor Staging

Most survival rate is tumor staging "by assessing the primary tumor size and cervical lymph node status." It should be noted that TNM staging alone cannot predict prognosis. Other tumor characteristics particularly histologic parameters must be utilized to identify the prognosis and select favorable treatment.

### Locoregional Recurrence (LRR)

Type of treatment, the presence of lymphovascular permeation, and observation of malignant cells microscopically in muscle, excluding "the extrinsic muscles of the tongue, pterygoid, and master muscles" can affect LRR.

### Lymph Node Metastasis

Several studies have indicated the involvement of cervical lymph node in OSCC patients. Regarding the role of lymph node metastasis in prognosis, radical neck dissection is performed when patients present with palpable (N+) cervical lymphadenopathy.

### Histologic Grading

The histologic grading is used to predict the clinical behavior of OSCC for many decades, but its prognostic value is still controversial. Anneroth's classification considers the degree of cell differentiation and keratinization, as well as pattern and stage of invasion, and

lymphoplasmocytic infiltration. Degree of cell differentiation, keratinization, and pattern of invasion correlate with survival rate, and among OSCC patients; the pattern of invasion is an independent prognostic factor of survival rate and lymph node metastasis. It is found that there is 44% decrease in survival rate per grade in OSCC. showed 44% decrease in survival rate per grade in OSCC.[4,5]

### Perineural invasion

Perineural invasion correlates with larger tumor size, higher depth of tumor invasion, risk of nodal metastasis, and lower 5-year survival rates in patient with OSCC.

### Surgical margins

Pathologic positive margin has been proven to be an adverse prognostic factor for OSCC patients, which apparently correlates with local recurrence and overall survival. The 5-year OS in early-stage OSCC patients with safe margin, positive margin, and close margin has been reported 78.2%, 61.4%, and 50.8%, respectively. Surgical clear margins > 5 mm are recommended to prevent local recurrence.

### Extracapsular spread (ECS) and depth of invasion

ECS de-extranodal extension of metastatic deposit outside the lymph node correlation between ECS and lower OS and decreasing survival rate between 29% and 60% when ECS is present. Liao *et al*. found that tumor thickness <10 mm is an independent prognostic factor for increasing OS and disease survival factor.

## GENETIC ALTERATIONS AND MOLECULAR BIOMARKERS OF OSCC

### Genetic Alterations

OSCC, like most other malignancies, arises from the accumulation of a number of discrete genetic events that lead to invasive cancer. These changes occur in genes that encode for proteins, which control the cell cycle, cell survival, cell migration, and angiogenesis.[6-8]

Previous cytogenetic analysis has shown a series of alteration in OSCC, most frequently in chromosome 9, chromosome 17 gene as well as 3P, 13q21, and 18q21. Correlation between hypermethylation of TP73, PIK3R5, and CELSR3, 42 down-regulation of MYC, 43 SMAD3/TGFBR2 genes mutation, am-gene, and survival rate in OSCC patients have been reported. Moreover, proteomic analysis of OSCC specimen revealed the correlation of 13 RNAs with their encoded proteins implying transcription control with survival rate. Among these, reduction of DSP, PKP1,

and TRIM is directly related to poorer disease-specific survival.[9]

Cancers including head-and-neck SCC (HNSCC) arise from the accumulation of genetic and epigenetic changes along with abnormalities in cancer-associated signaling pathways mentioned by Hannah Weinberg. These include:

1. Limitless replicative potential of tumors
2. Self-Sufficiency in growth signals
3. Insensitivity to anti-growth signals
4. Evade apoptosis
5. Increased angiogenesis and
6. **Invasion and metastasis.**

## THE CELLULAR MICROENVIRONMENT OF HNSCC

### Cancer-associated Fibroblasts (CAFs)

Commonly activated in tumors, these activated fibroblasts, termed CAFs, share many similarities with activated fibroblasts or myofibroblasts found in wounds and in inflammatory sites. CAFs are the most abundant cells of the tumor microenvironment. CAFs are usually recognized by the expression of α-smooth muscle actin, similar to myofibroblasts present at the site of wound healing and chronic inflammation, which is absent in normal dermal fibroblasts. CAFs might differentiate locally from normal stromal fibroblasts of surrounding tissue or from bone marrow-derived mesenchymal stem cells recruited to the tumor.[10-13]

### Tumor-Associated Macrophages

Infiltrating inflammatory cells in HNSCC include monocytes, dendritic cells, and macrophages, which are normally present in a variety of reactive inflammatory lesions. The association between the bacterial profile and OSCC has a been studied. The numbers of *Porphyromonas* spp., *Fusobacterium* spp., and the bacterial species were significantly higher in the OSCC tissue than in adjacent healthy mucosa. Using immunohistochemical staining, it has been shown that the number of *Porphyromonas gingivalis* bacteria in gingival SCC was higher than that in healthy gingival tissue samples. Moreover, numerous live oral bacteria were detected in metastatic lymph nodes of oral cancer patients more frequently than in uninvolved nodes, suggesting that the colonization by these bacteria may be associated with oral cancer.[14,15]

### Tumor Infiltrating Lymphocytes

Almost 20 years ago, human tumour was described as "wounds that do not heal." Indeed, changes occurring in the microenvironment of the progressing tumor

resemble the process of chronic inflammation, which begins with ischemia followed by interstitial and cellular edema, appearance of immune cells, and finally, growth of blood vessels and tissue repair.[16-18]

T lymphocytes are the gatekeepers of autoimmune regulation. Failure of T lymphocytes to recognize and eradicate malignant cells contributes to tumor development. Tumors with a high infiltrate of lymphocytes are associated with improved prognosis. HNSCC tumors are influenced by several classes of T lymphocytes including T-helper cells, CD3, 4 or 8 positive T cells, natural killer cells, regulatory T cells, and myeloid progenitor cells.

Depending on the subtype of T cells infiltrating the tumor, the tumor experiences growth promotion or regression.[19]

## ENDOTHELIAL CELLS

Endothelial cells when stimulated by the growth factors form blood vessels that facilitate tumor growth and dissemination. HNSCC cells directly bind to endothelial cells through adhesion molecules including intercellular cell adhesion molecule-1, CD44, lymphocyte function-associated antigen-3, and integrin chains.[20,21]

Direct binding of HNSCC to endothelial cells is a prerequisite for the penetration of metastasis through the vasculature. In addition, direct interaction between HNSCC and endothelial cells triggers Notch-1 signaling in endothelial cells promoting capillary tubule formation.[22]

## LYMPHATIC CELLS, PERICYTES, MAST CELLS, AND OTHER CELLS IN THE TUMOR MICROENVIRONMENT

In addition to blood vessels, HNSCC is typically infiltrated by lymphatic vessels, a process known as lymphangiogenesis. Lymph vessels are typically distributed throughout the tumor as well as in the peritumoral regions. Metastasis to regional lymph nodes commonly occurs in HNSCC and correlates with poor prognosis.[23-25]

Pericytes are contractile stromal cells closely associated with vascular endothelial cells that stabilize the capillary walls. In the absence of pericytes, blood vessels are unstable and undergo regression. Pericytes influence the proliferation, migration, and maturation of endothelial cells. In tumors, pericytes are loosely associated with endothelial cells resulting in increased capillary leakiness.

Mast cells are white blood cells that directly associate with endothelial cells stimulating vascular tube formation. As HNSCC progresses, there is an increase in mast cell numbers that correlated with angiogenesis suggesting a role in angiogenesis.

The oral cavity and associated areas of the head and neck region are exposed to several microorganisms. Metaproteomic analyses of human salivary microbiota revealed a large number of oral bacteria that are metabolically active and actively engaged in protein synthesis. The role of the human oral microbiome in tumor pathogenesis remains largely unknown. It is well known that bacteria associated with periodontitis, a condition caused by chronic inflammation of the gums, poses an independent risk factor for HNSCC. HPV infection is a major risk factor for oropharyngeal SCC.[26]

## THE CELLULAR MICROENVIRONMENT OF HNSCC

Although effective antitumor immune responses likely involve many components of the immune system, T-cells continue to be considered as the critical immune cells involved in antitumor immunity. T lymphocytes are considered an essential component of antitumor immunity, with CD8+ T cells serving as cytotoxic effector cells and CD4+ Th1 cells serving to "help" and enhance the magnitude and duration of the antitumor responses. However, CD4+ Th2 cells and CD4+ T regulatory cells are capable of suppressing effective CD8+ antitumor responses. Several investigators have found dysfunctional circulating and tumor-infiltrating T cells in HNSCC patients, with functional assays identifying multiple defects in T-cell activation and effector function, suggesting that the tumor has successfully suppressed an otherwise robust lymphocytic response patterns of tumor-related leukocyte infiltration varying between primary tumors and metastatic lymph nodes in HNSCC with a local decrease in the number of CD8+ T-cells and increase in CD20+ B-cells.[23,27]

## CONCLUSION

A deeper understanding of the factors that cause immune suppression in OSCCs might be relevant for the development of novel anticancer therapies. The worse prognosis of these patients has been linked to hypoxia and hypoxia-induced immune escape. Impaired anti-tumor responses of OSCC patients are caused not only by the tumor itself and by the presence of functional defects or apoptosis of both circulating and tumor-infiltrating T cells but also by soluble factors of the tumor microenvironment including soluble factors and the hypoxic microenvironment, which leads to an accumulation of immune suppressive cells, like TAM, Tregs, and MDSCs macrophages as well as a downregulation in the function and activity of T lymphocytes and DCs.

However, the mechanisms by which changes in stromal cells facilitate HNSCC growth are not completely understood at present. Increased understanding of the mechanisms involved in the complex crosstalk between cells and the tumor microenvironment hold great promise for designing strategies to target HNSCC effectively.

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