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REVIEW

Tumor Microenvironment in Head and Neck Squamous Cell Carcinoma: A Focus on Tumor-Infiltrating Lymphocytes

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Abstract

For more progress in head and neck squamous cell carcinoma (HNSCC) immuno-oncology, further understanding of interactions between tumor and immune system as well as factors in the tumor microenvironment is required. HNSCC is seriously infiltrated by lymphocytes but is known to be highly immunosuppressive. The aim of this review is to highlight the complexity of tumor microenvironment and tumor- immune cells interaction in the HNSCC, in order to improve understanding of tumorigenesis and disease progression in HNSCC patient and to provide valuable information about prognostic markers. The main goal of this review is to discuss the role of the tumor infiltrating lymphocytes in tumor progression, their cross-talk with other components of the tumor microenvironment as well as their roles in carcinogenesis, metastasis process, treatment, and prognosis in head and neck squamous cell carcinomas.

Keywords: HNSCC- tumor infiltrating lymphocytes- tumor microenvironment- lymphocytes- inflammation- hypoxia

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous group of upper aerodigestive tract malignancies that accounts for 90% of all head and neck cancer cases. HNSCC is the sixth most common cancer by incidence, and a leading cause of cancer-related death [1]. Besides alcohol and smoking, human papillomavirus (HPV) has been recently regarded as a major etiological factor for a subgroup of HNSCC including oropharynx [2-5].

Despite the improvement of treatment regimens, the 5-year survival of HNSCC patients is only about 40-50% [6]. Tumor micrometastases to lymphovascular and/or perineural are associated with increased recurrence rate [7]. Accordingly, the development of new treatment modalities and establishing accurate prognostic markers is always demand.

Recently it has been shown that tumor aggressiveness and therapy resistance are extremely influenced by the interactions between tumor cells and their surrounding microenvironment [8-9].

HNSCC tumors are highly infiltrated by different types of immune cells which are considered as important elements of TME for predicting clinical outcome of the disease [10-13]. Although the presence of certain immune cells in the tumor microenvironment (TME) is related to favorable outcome [14], some indications propose that antitumor immune responses are impaired in HNSCC and are related to disease progression [15].

Different aspects of the HNSCC tumor microenvironment including the influence of tumor cells-immune cells interactions on the activation and regulation of cell-mediated immune responses have remained to be cleared. Comprehensive understanding of the interactions between immune cells and tumor microenvironment components in HNSCC may lead to the development of new cancer therapy modalities to improve immune responses against this highly mortal malignancy.

The purpose of this article is to review the main components of the tumor microenvironment in HNSCC, their cross talk and the effect of metabolic changes on the structure/ function of the tumor microenvironment.

An overview of the tumor microenvironment in HNSCC

The tumor microenvironment is a complex and dynamic network of cellular and non-cellular components

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including malignant cells, cancer-associated fibroblasts, endothelial cells, infiltrating immune cells, and secretory mediators such as exosomes which contribute to the establishment of complex crosstalk with the tumor entity [16-17]. It has been shown that some tumor microenvironment-associated factors such as hypoxia [18], inflammation and angiogenesis [19] may influence the infiltration of lymphocytes to HNSCC and play pivotal roles in the tumor development, invasion and metastasis. Environmental factors such as tobacco or alcohol have been shown to influence the progression of HNSCC through the induction of genetic alterations, which in turn result in the suppression of immune system, the transformation of stromal cells and induction of chronic inflammation [8-9].

As a highly immunomodulatory tumor, HNSCC takes advantage of genetic and environmental mechanisms such as the selection of poorly immunogenic cancer cell subsets, production of proinflammatory and immunosuppressive cytokines, secretion of exosomes, induction of immunosuppressive immune cells and expression of immune checkpoint pathway molecules [20-24]. The mechanism by which tumor infiltrating cells become suppressed in the tumor microenvironment is not completely understood, though it has been associated with the presence of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells (MDSCs) [25-26]. Recent studies have also shown that the release of tumor-derived exosomes is related to the immunosuppression in the tumor microenvironment [27].

Exosomes are small membranous vesicles originated from endocytic compartments which are released to the extracellular spaces. Exosomes have recently been identified in several human malignancies including melanoma [28], Glioblastoma [29], colorectal cancer [30] and Hepatocellular cancer [31]. Tumor-derived exosomes are composed of different proteins and microRNAs and play a key role in the cross-communication between tumors and the cells of the immune system, promoting the immune evasion of tumors [23-32-35]. The presence of Exosomes, in HNSCC and some other tumors, has been correlated with advanced tumor stages. Exosomes have been considered as main contributors responsible for progression, metastasis, survival, immune regulation and other invasive characteristics of tumors which function through communication with cells in the tumor microenvironment. Although the underlying mechanisms of the production of exosomes are yet to be explored, it has been shown that exosomes act as carriers for immunosuppressive molecules delivering them to the target immune cells. Recent studies have shown that increased levels of immunomodulating PD-L1+exosomes isolated from blood samples of HNSCC patients are linked to tumor progression and immune suppression [36-37].

It has been shown that exosomes have different morphology and molecular features in oral cancer patients compared to healthy individuals, which can be used as an early diagnostic marker for identifying malignant changes in high-risk cases [38]. In the tumor microenvironment, exosomes through interaction with hypoxia, inflammation, and angiogenesis associated elements can influence the incidence and progression of tumors. In hypoxic microenvironment tumor cells stimulate generate miR-21-rich exosomes that are delivered to normoxic cells and promoted prometastatic behaviors. Moreover, exosomes derived from hypoxic oral squamous cell carcinoma (OSCC) cells increase the migration and invasion of tumor cells in a HIF-1α and HIF-2α-dependent manner [39].

The effect of Hypoxia, inflammation in head and neck squamous cell carcinoma microenvironment on tumor infiltrating lymphocytes

In many types of tumors including HNSCC, the inflamed microenvironment in combination with low oxygen concentration is ideal factors which contribute to tumorigenesis and angiogenesis through immune cell dysregulation such as apoptosis of cytotoxic T cells and activation of suppressor T cells [40-42]. Hypoxia inducible factor (HIF) is a transcription factor which is induced following hypoxia in the tumor microenvironment. HIF acts as a regulator of immune cells effector function through an effect on their cytokine production ability, their survival, and apoptosis [43]. It was shown that high PD-L1 expression by the tumor cells under hypoxic conditions, which induces tumor resistance into CD8+ T cells toxic agents [44]. In addition, HIFs display strong signaling which results in the switch of inflammatory responses to a pro-tumorigenic state by recruiting immune cells and changing their effector functions to suppress antitumor immune responses. Higher expression of HIF is reported as a negative prognosticator in patients with HNSCC [45]. It has been reported that tumor infiltrating lymphocytes in HNSCC, which encountered low oxygen concentration, reduce production level of cytokines and granzyme due to the hindrance of Kv1.3 channels [46].

Inflammation, as the second factor which contributes to tumor growth, has been shown to closely relate with HNSCC development. In a murine model of HNSCC it has been observed that in premalignant stage, the level of Th1, Tc1, and Th17 increased in comparison with a control group and HNSCC-bearing mice, however, the frequency of regulatory T cells was higher in HNSCC-bearing mice [47]. Another study showed that in established HNSCC, lower levels of proinflammatory agents were detected including CCL5, MCP-1, G-CSF, and PGE2 as compared with lesions of premalignant state [48]. These two above mentioned studies suggest the premalignant microenvironment to be more immune stimulatory than the microenvironment of an established HNSCC. However, it remains uncertain if inflammation at the primary tumor site represents a beneficial manifestation of a patient's immune response to cancer in the tumor microenvironment or actually is a carcinogenic response that enhances tumor progression through the elaboration of regulatory cytokines such as IL-6, IL-10, and IL-17 [49-51]. Further investigations were needed in order to understand the multilateral effects of

the inflammation at the primary state of the tumor.

TILs in HNSCC

Tumor-infiltrating lymphocytes (TILs) are important predictors of tumor biology and outcome. Several studies showed the presence of TILs in different tumors and functional subsets are a favorable prognostic factor for treatment and linked with patients' clinical outcomes [52-53].

In HNSCCs, better response to definitive chemo radiotherapy has been reported in cases with a higher number of TILs [54-55], and better outcomes were reported following surgery with adjuvant therapy [56].

Presence of TILs in HNSCC indicates that this cancer can be considered as immunogenic cancer. However, the antitumor immune responses are affected by functional defects or apoptosis of both circulating and tumor-infiltrating T-cells [15-57-61]. Moreover, numbers of TILs, their function, and location in the HNSCC microenvironment may significantly vary independent ly to the site and size of tumors. For example, oropharyngeal tumors contain higher levels of T-cell infiltration, compared to tumors at other sites of the head and neck [62-63].

Studies have shown that different subsets of lymphocytes have altered or even opposite functions in the tumor microenvironment. Indeed, it has been founded that high CD8+ T cell infiltration associated with a better prognostic value and outcome in HNSCC [64]. however, the role of a wide range of CD4+ cell subsets with different functions in the tumor microenvironment and their prognostic role remains to be elucidated [65-66].

T cells

T cells are one of the important factors that organize the immune system to check and remove malignant cells in favor of immune surveillance [67-68]. In several malignancies such as colorectal and ovarian cancer, high infiltration of CD8+ Cytotoxic T cells are positively correlated with prognosis and favorable outcome compared to no infiltration [69-73]. Also, it has been reported patients with HNSCC whose tumor was extremely infiltrated by CD8+ T cells have a significantly better outcome compared to patients with slight or no infiltration of CD8+ T cells [44-55-56-74-75]. However, in the study of oral cavity tumors, high level of CD8+ TILs has shown no significant prognostic value [76-78] or was positively correlated with tumor recurrence [79]. These results could be a consequence of different biological behavior oral cavity with a high grade of local invasion and metastasis to the cervical lymph nodes [80].

In addition, some evidence showed differences in prognostic significance of TILs depending on the tumor compartments (tumor epithelium, tumor stroma). For instance, in HPV-positive oropharyngeal squamous patients, stromal infiltration of CD8 T cells was associated with favorable outcome [81]. Other studies suggested that a high level of CD8+ cells in tumor epithelium predicted a better clinical outcome [64]. However, in a study conducted by Balermpas et. al the differences in the prognostic value of stromal and epithelial CD8+ cells has not been reported [56]. It seems that more studies are needed to shed light on the role of CD8+ cells in different compartments of the tumor and their impact on cancer prognosis.

However, the advantage of CD4+ T cell infiltration in the tumor microenvironment is slightly controversial. At first, CD4+T cells were found as a positive prognostic factor in pancreatic and esophageal squamous cell carcinoma. In HNSCCs, studies suggested that high levels of tumor-infiltrating CD4+CD69+ T cells were positively correlated with more favorable prognosis [76-82]. In contrast, other studies proposed CD4+T cells as a poor prognostic predictor, especially in oral cavity cancer [83]. Heterogeneity of the CD4+T cell population and its characteristic cytokine profiles may explain these contradictory results [84].

In the microenvironment of many solid tumors, such as hepatocellular, breast and lung cancer, a high ratio of cytotoxic CD8+ T lymphocytes/Treg cells was reported as a good prognostic factor [14]. However, the prognostic value of Treg cells seems to be different between types of cancer. Previous studies suggested that in HNSCC high immune-suppressing Treg cells number was associated with better prognosis and clinical outcome [85]. In a study on the tumor specimens of oral cavity cancer patients, low stromal cytotoxic CD8+ T-lymphocyte counts and the high number of stromal Treg cells were associated with low survival [86]. Some studies on oropharynx cancer showed different results compared with other types of head and neck cancers [87-89]. The data show that in oropharyngeal cancer high levels of cytotoxic CD8+ T cells could be considered as a positive prognostic factor, but the influence of high rate of T regulatory cells is controversial. According to this, more studies are required to confirm the prognostic value of the CD8+ T lymphocytes/Treg cells ratio in HNSCC.

B cells

Most former studies have focused on cytotoxic T cells that exhibit the highest antitumor activity among other immune cells. Recently B cells, another important fraction of TILs, have been identified as the important predictor of disease outcome [90-92]. Evidence shows that B cells can promote or inhibit the progression of tumors through producing antibodies against the tumor antigens, acting as APC and secreting numerous cytokines. Several factors such as tumor type and the subset of B cell influence the role of B cells in the support or prevention of tumor growth.

Most previous studies in HNSCC have investigated B cells by immunohistochemistry method that prognostic effect of tumor-infiltrating CD20+ B cells was described on the outcome [93-94]. Distel et.al showed a favorable outcome correlated in the high percentage of B-lymphocytes in patients with early-stage HNSCC, in contrast to an inverse association in the advanced stage. These results suggest that there is phenotypic and functional plasticity of B cells during the course of disease progression [95]. However knowledge about the different B cell subsets in

tumor microenvironment of HNSCC is in short, recent study of flow cytometric analysis of tumor-infiltrating B cell subpopulations including activated ($CD86^+$), antigen-presenting ($CD86^+CD21^-$), memory (IgD^-CD27^+) in HNSCC demonstrated significant difference in the frequencies of different B cell subsets in tumor microenvironment of patients with HPV+ compared to HPV- HNSCC although it did not show any relation to disease stage [96].

Besides, in some few studies infiltration of HNSCC by regulatory B cells was investigated. Lechner et. al observed the high infiltration of $CD24^{hi}CD38^{hi}$ and $CD25^{hi}$ regulatory B cells in tumor tissue of HNSCC compared to peripheral blood of patients and healthy controls [97]. However, the significance of regulatory B cells in immunity against tumor remains to be elusive in humans. In tongue squamous cell carcinoma has been indicated that CD19+IL-10+ regulatory B cells affected the survival of patients by inducing Tregs through secretion of IL-10 [94]. Still, further investigation is required to clarify in more detail function and phenotype of regulatory B cell subsets in the tumor microenvironment of HNSCC.

Immune-inflamed cancer phenotype and benefit to immunotherapy

Like other solid tumors, HNSCC shows two main immunophenotypes: i) inflamed tumor type with a rich T cell infiltrate, a type 1 interferon signature, and various chemokine profiles, ii) non-inflamed type without these structures [98]. The structure of T cell- inflamed tumor indicated a previous anti-tumor immune response might have existed that was ineffective due to the obstruction of tumor penetration through stroma or by the retention of immune cells in the stroma [99]. Understanding the resistance mechanisms in both T cell–inflamed and non-inflamed tumors are essential for overcoming treatment failure and increasing the response rate of patients to current immunotherapy. In addition, the modulation of the tumor microenvironment has become increasingly an issue in the field of immunization, and studies on immune checkpoint mediated immunosuppression and cancer immunotherapy have peaked the safety of cancer treatment [100].

The new mAbs approved by the US Food and Drug Administration (FDA) for HNSCC patients are anti-PD-1 mAbs nivolumab and pembrolizumab [101], in addition to cetuximab, a mAb against epidermal growth factor receptor (EGFR) [102]. However, most do not benefit from anti-PD1 therapy. Understanding of Inhibitory checkpoint receptor mechanisms may help the clinician to correctly select certain immunotherapy options for specific patients. Some immune checkpoints such as LAG3 and TIM3have become attractive goals for prevailing to the resistance of tumors with an inflamed phenotype including melanoma, NSCLC, and HNSCC [103]. Due to the potentially severe toxicity and high costs of immune checkpoint inhibitors, the search for predictive biomarkers that can target immune cell infiltration is highly demanded. The immune cell subsets and their position in the TME could affect the prognosis and prediction of response to Immune-checkpoint inhibitors therapy. Besides, Most clinical trials in both recurrent and metastatic HNSCC patients indicated that factors other than PD-L1 expression, including tumor-immune cell infiltration, tumor mutational burden and human papillomavirus (HPV) may contribute to the patients' response to treatment [63-104].

Taken together, a better understanding of the tumor-immune cell cross talk and the resistance mechanisms in both T cell–inflamed and non-inflamed tumors, affect the successfulness of immunotherapy for overcoming resistance to available therapies and designing novel immunotherapies in order to increase patients benefit from immunotherapy.

In conclusion, HNSCC tumor microenvironment is composed of stromal fibroblasts, vasculature, immune cells, cytokines, and hypoxia which play a supportive role in the initiation, progression, and metastasis of the tumor. In HNSCC, there is a complexity in tumor-immune system interactions which are mainly dictated by the tumor microenvironment. A rational approach to further clinical investigation requires a deeper understanding of the interaction of the immune system with the tumor microenvironment. As more is discovered about the interaction of the immune system with HNSCC tumors in the development of the disease and in the mechanisms of tumor resistance; the opportunity for earlier intervention may also become possible.

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