Review

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The role of cytokines in head and neck squamous cell carcinoma: A review

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, accounting for approximately 6% of all cancer cases and responsible for an estimated 1-2% of all cancer deaths. Much research evidence has accumulated in the recent years on the changes in the expression of pro-inflammatory and, to a lesser extent, anti-inflammatory cytokines, that (i) may have a role in the malignant transformation of HNSCC, (ii) may be used as diagnostic markers in the sera of patients because of their excessive production by the tumor cells and (iii) may act as possible immunotherapeutic targets. Among pro-inflammatory cytokines, interleukin-8 (IL--8) has been reported to have an important role in cancer invasion, angiogenesis and metastasis. Recent studies have shown an increased concentration of IL--8 in patients with HNSCC and a positive association with lymph node metastasis and tumor classification, although IL--8 was not significantly associated with shorter overall survival and cancer progression-free survival. Additional evidence on the pathological mechanism of origin, invasion, and metastasis of HNSCC, as well as a better understanding of the implications of cytokines, chemokines and growth factors, are of paramount importance for the advancement of research in head and neck oncology. ***Clin Ter 2020; 171 (3):e??-??. doi: 10.7417/CT.2020.????***

Key words: *head and neck cancer, cytokines, chemokines, interleukin-8, squamous cell carcinoma*

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, accounting for approximately 6% of all cancer cases and responsible for an estimated 1-2% of all cancer deaths. Oral squamous cell carcinoma and laryngeal squamous cell carcinoma (LSCC) are the most common head and neck cancers globally, with an age-adjusted standardized incidence rate of 3.9 and 2.3 per 100.000, respectively.

Several risk factors have been implicated in the pathogenesis of head and neck cancer; the most significant are tobacco and alcohol consumption (1) that act synergistically, resulting in an approximately 35-fold increase in HNSCC risk in heavy smokers (>2 packs/day) and drinkers (>4 drinks/day). In particular, the metabolism of alcohol is regulated by specific enzymes whose activity and expression is influenced by genetic polymorphisms playing key roles in the development of cancer (2, 3). In addition, exposure to other environmental factors is thought to potentially increase the risk of HNSCC, such as asbestos, polycyclic aromatic hydrocarbons, and textile dust (4). Dietary factors have also been noted, with red meat increasing the risk of head and neck cancer, while a diet varied in fruit and vegetables potentially has a protective effect. The controversial role that both gastroesophageal and laryngopharyngeal reflux play in the disease process is also under investigation.

In the recent years, an increasing pathogenetic role has been demonstrated for the human papillomavirus (HPV), a proven driver of most tumors of the oropharynx (5-13). HPV-positive cancers differ from HPV-negative HNSCC, as patients with HPV-related HNSCC are younger and report a lower consumption of tobacco and alcohol and are often diagnosed at a later stage. HPV-positive HNSCC show an affinity for the oropharynx and have a better prognosis regardless of the treatment regimen compared with HPV-negative HNSCC (5-10, 14-16).

Over the past two decades, even though patients have benefited greatly from the latest advances in surgical techniques, chemotherapy and radiation therapy, the overall survival rate of HNSCC has not improved significantly (17-20). Furthermore, TNM classification is not sufficient for the estimation of tumor aggressiveness and the heterogeneous group of investigated HNSCC cancers in different locations such as oral cancers, laryngopharyngeal carcinomas, nasal cavities and paranasal sinuses tumors may lead to different conclusions.

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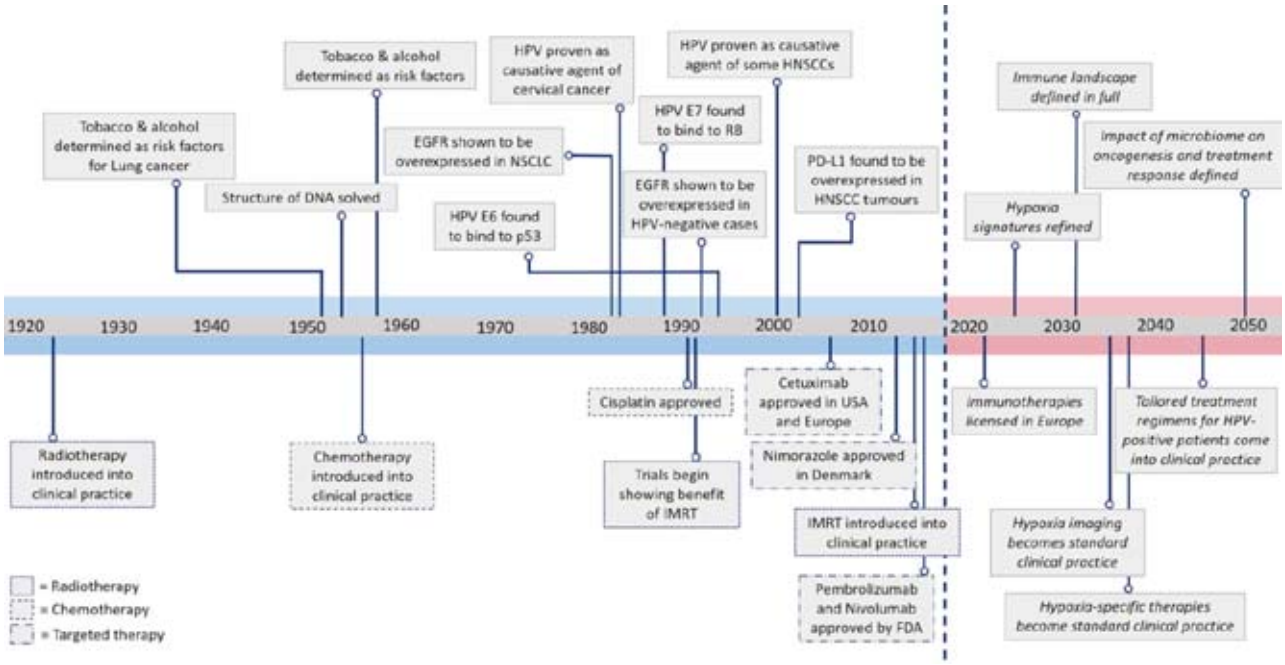

Fig. 1. Timeline of the innovations in head and neck cancers and possible future directions. The main advances in the understanding of HNSCC have been made in the past 2–3 decades. From (21)

Most of the developments towards understanding this disease have occurred in the past two decades but have fallen short of clinically meaningful discoveries. Fig. 1 shows a timeline of the innovations in head and neck cancers and possible future directions (21).

It has been reported that changes in the expression of cytokines, chemokines and growth factors may have implications in the malignant transformation of many cancers (22-25) including HNSCC and, more recently, LSCC (17, 26-28). In a recent study from Fallahi et al (29) on the role of cytokines and chemokines in papillary thyroid cancer, the authors demonstrated that that (C-X-C motif) ligand 9 and 11 chemokines were absent basally in non-neoplastic thyroid and papillary thyroid carcinoma cells. Interferon (IFN)γ induced the chemokine secretion in both conditions, while tumor necrosis factor (TNF)α induced it only in papillary thyroid carcinoma.

In this review, the authors will briefly discuss the role of cytokines in head and neck cancer, with a special focus on Interleukin (IL)-8, a pro-inflammatory cytokine that has recently been reported to play an important role in HNSCC cancer invasion, angiogenesis and metastasis.

Cytokine classification

Cytokines are a group of soluble proteins with low-molecular-weight able to mediate the immune and inflammatory responses. Cytokines can be classified based on their biological properties into three groups: T-helper 1 (Th1), T-helper 2 (Th2) and T-helper 17 (Th17) (30). Th1 cytokines stimulate cellular immune responses, while Th2 cytokines predominantly regulate humoral responses. Th17 is currently known to regulate inflammatory responses and plays several roles in autoimmunity (30). Cytokines can also be classified according to their action on inflammation as pro-inflammatory or anti-inflammatory (31). Pro-inflammatory cytokines are a group of immunoregulatory cytokines that favor inflammation. Pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. They include IL1-alpha, IL1-β, IL-6, and TNF-alpha. Other pro-inflammatory mediators include members of the IL-20 family, IL-33 IFN-gamma, granulocyte macrophage-colony-stimulating factor (GM-CSF), TGF-β, IL-11, IL-12, IL-17, IL-18, IL-8 and a variety of other chemokines that chemoattract inflammatory cells (32, 33). Anti-inflammatory cytokines control the pro-inflammatory cytokines response by acting in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Major anti-inflammatory cytokines are IL-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13. Leukemia inhibitory factor, interferon-alpha, IL-6, and transforming growth factor (TGF)-β are categorized as either anti-inflammatory or pro-inflammatory cytokines, under various circumstances (34, 35).

As for Nerve Growth Factor (NGF), several studies led to divergent hypotheses about the role of NGF, its specific distribution pattern within the tissues and its implication in induction as well as progression of carcinogenesis. However, other recent studies have shown that NGF may have direct clinical relevance in certain tumor cell prevention (36-40).

Cytokines in head and neck squamous cell carcinoma

Recent studies have investigated the involvement of cytokines in the pathogenesis of HNSCC (41-44) categorizing cytokines as (i) factors that affect tumor growth, (ii) factors that can be used as prognostic markers and (iii) those that are

possible immunotherapeutic targets (45). Although the main source of cytokines are immune cells, many tumor cells have been shown to make autocrine mediators to support their own growth thus evading the immune response (46); they include head and neck carcinomas that produce IL-4, IL-6, IL-8, IL-10, GM-CSF, VEGF, prostaglandin E2 (PGE2) as well as basic fibroblast growth factor (bFGF) (47-51).

A list of relevant cytokines for HNSCC is shown in Table 1 (Table 1).

Some of these cytokines may be used as additional diagnostic markers in the sera of HNSCC patients because of their excessive production by the tumor cells (53); this could be valuable on current research on therapeutic strategies since there are currently no reliable markers to predict either tumor development or relapse in treated HNSCC patients.

Recent evidences have associated HNSCC development to a decrease in Th1 and an increase in Th2 cytokine levels, a mechanism to evade anti-tumor immune response and affect tumor growth (54, 55). This shift towards the Th2 cytokine response is a common event in many other solid tumors, such as colorectal cancer, renal cell carcinoma, prostate cancer, and melanoma (56), and is also valid for HNSCC. Bleotu et al found (57) a clear switch from cytokine Th1 to cytokine Th2 in HNSCC patients, low levels of IL-2 and IFN-γ in advanced stages, as well as a positive correlation of increased levels of both IL-2 and IL-12 with the early stages of laryngo-pharyngeal cancer. Loco-regional metastases were correlated with increased levels of IL-8 and IL-10 and drastic decrease of IFN-γ. In advanced cancer stages, the authors found that the most affected were IL-2 and IFN-γ correlated with increased levels of Th2 cytokines, supporting the hypothesis that the ratio between different Th1 and Th2 cytokines could represent a useful marker for clinical and pathological evaluation of cancer patients. Furthermore, this evidence could be of great value to develop immunotherapeutic approaches to cancer that aim to shift the balance in favor of Th1 response (58-61).

Among pro-inflammatory cytokines, IL-8, part of the CXC chemokine family that was originally classified as neutrophil chemoattractant, has attracted much recent research efforts and is now reported to play an important role in cancer invasion, angiogenesis and metastasis (62-67). It has been demonstrated in several cancers, such as in breast cancer, gastric cancer, colon cancer, cervical cancer, pancreatic cancer and leukemia, that the cancer cells themselves can also secret IL-8 in an autocrine or paracrine manner (68). In human colon cancer cell lines, constitutive expression of IL-8 has been linked to metastatic potential and has been suggested to play a role in the development of distant metastases. In vivo analysis also showed that IL-8 would be a sensitive marker in predicting prognosis and monitoring disease progression of the pancreatic cancer patients (68).

IL-8, along with other factors produced either by normal or malignant cells such as VEGF and FGF, has been shown to contribute to tumorigenesis, metastasis and angiogenesis in patients with HNSCC (17, 41, 69). Angiogenesis, indeed, is one of the factors that is known to positively drive metastasis and it has been reported to be associated with decreased survival of HNSCC patients (17, 49, 70, 71).

IL-8 production is linked with tumor vascularization, metastatic phenotype, tumor growth, and overall poor prognosis; serum levels of IL-8 have been found to be consistently

Table 1. HNSCC relevant cytokines and their proposed cellular functions

Cytokine	Sites of action
Basic fibroblast growth factor (bFGF)	Angiogenesis, metastasis
Granulocyte macrophage-colony-stimulating factor (GM-CSF)	CD34 mobilisation, immune suppression
IL-1	Cytokine secretion, gelantine production
IL-4	Immune suppression
IL-6	Inflammation regulation, anti-apoptosis
IL-8	Angiogenesis
IL-10	Immune suppression
Hepatocyte growth factor (HGF)	Angiogenesis
Macrophage migration inhibitory factor (MIF)	Growth regulation
Platelet-derived growth factor (PDGF)	Angiogenesis
Prostaglandin E2 (PGE2)	Immune suppression
Transforming growth factor-σ (TGF-β)	Immune suppression
Vascular endothelial growth factor (VEGF)	Angiogenesis, metastasis, chemoattraction

elevated in patients with recurrent or metastatic HNSCC and increasing evidence is correlating elevated IL-8 levels with advanced or aggressive disease (42, 44, 72).

Linkov et al (41) and Hoffmann et al (46) reported an increase in IL-8 concentration in patients with HNSCC, although with limited statistical significance. Similarly, Gokhale et al (72) reported that IL-8 was not elevated in patients with a new diagnosis of HNSCC but was elevated in patients with disease recurrence or metastatic HNSCC.

A study of Li et al (73) showed that IL-8 can be significantly triggered by SDF-1/CXCR4 interaction in HNSCC and demonstrated that IL-8 secretion mechanism is regulated by Akt phosphorylation after SDF-1 stimulation. These results point out the importance of SDF-1/CXCR4 interaction in HNSCC angiogenesis and provides a new targeting therapy utility, disrupting SDF-1/CXCR4 interaction combined with downstream-induced angiogenic factors in HNSCC would be beneficial to improve clinical outcome.

Swenson et al (74) demonstrated that IL-8 and VEGF expression is based on interactions between NF-kB, AP-1, and NF IL-6. The authors identified at least 1.5-fold dose-dependent induction of AP-1, VEGF, and IL-8 promoter/reporter gene activity after 24-hour exposure to cigarette smoke condensate reporting that tobacco carcinogens up-regulate AP-1 activity and AP-1 dependent IL-8 and VEGF gene expression in head and neck cancer (74). This up-regulation may promote an angiogenic phenotype that favors invasion in both premalignant and squamous cancer cells of the head and neck. Cigarette smoke condensate could therefore significantly stimulate AP-1 activation of both genes, resulting in increased IL-8 and VEGF secretion, and these processes could be down-regulated with introduction of a dominant negative A-Fos gene (74). These data demonstrate a role for tobacco carcinogen stimulation of pro-angiogenic cytokines, thus promoting an environment suitable for development and metastatic spread of head and neck cancer cells.

The role of IL-8 in the development and progression of laryngeal cancer has also been recently investigated (75). In the last decade, four studies focused on the role of IL-8 in LSCC, enrolling a total of 220 patients with LSCC or dysplasia (57, 68, 76, 77). These studies demonstrated (i) some elevation, mostly associated with the tumor size, of IL-8 cytokine level in patients affected by laryngeal cancer (57, 68, 76); and (ii) the association of serum levels of IL-8 in patients with LSCC with lymph node metastasis and tumor classification (68). The increased levels of IL-8 in patients with locally-metastatic LSCC may confirm the importance of this cytokine as an indicator of the presence of local metastases, potentially contributing to the correct evaluation of patients and an adequate therapy selection. However, much controversy is still present on the topic and more research is needed to better elucidate the role of IL-8 in LSCC.

Increasing evidence has accumulated on the role in cancer progression of neutrophil-to-lymphocyte ratio (NLR), a marker of subclinical inflammation (78-82). An increased NLR is associated with poorer prognostic outcomes in numerous types of cancer, including HNSCC (83-86); however, a small number of studies have demonstrated the prognostic role of NLR in patients with LSCC (87). A study of Du et al (88) evaluated the association between NLR and survival outcomes in 654 patients with LSCC. In the study has been reported an association between clinical characteristics of the patients and blood and biochemical parameters (including NLR), platelet-to-lymphocyte ratio and albumin-to-globulin ratio, with the exception of histologic grade. Survival analysis demonstrated that NLR at cutoff values subdivided patients into different survival outcomes; subsequent to adjustments for age and other clinical features, NLR was identified to be an independent prognostic factor for overall survival and progression-free survival. Increased levels of cytokines, including IL-6 and IL-8, in tumor tissues were associated with NLR values.

Current knowledge on the role of cytokines in HNSCC has some limits, especially in the pathological role in cancer development, prevention and treatment. Much controversy still exists on this topic and more research is needed to better elucidate the pathological mechanisms and the intervention options.

Conclusions

One of the emergent and most promising scientific fields in head and neck cancer is actually the investigation of the mechanisms of origin, invasion, and metastasis of the cancer. Cytokines and growth factors may have implications in the malignant transformation of many cancers including HNSCC, as well as be used to monitor or predict tumor aggressiveness and as a target of immunotherapy. Evidence available in the literature is encouraging; however, more research is necessary to better elucidate the pathological role and future perspectives of cytokines in head and neck cancer development, prevention and treatment.

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