Chapter 5
The Role of Inflammation in Head and Neck Cancer

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Abstract Cancer-related inflammation is considered the "seventh hallmark of cancer"; numerous studies demonstrate that tumors develop and progress within inflammatory diseases. Central to the development of cancer are genetic changes that endow these cancer cells with many of the hallmarks of cancer, such as self-sufficient growth and resistance to anti-growth and pro-death signals. However, while the genetic changes that occur within cancer cells themselves, such as activated oncogenes or dysfunctional tumor suppressors, are responsible for many aspects of cancer development, they are not sufficient. Tumor promotion and progression are dependent on ancillary processes involving cells of the tumor environment that are not necessarily cancerous themselves. Infiltration of immune cells facilitates tumor development through the production of factors that promote carcinogenesis and by enabling tumors to evade the host immune response. Small molecules including cytokines, chemokines, and growth factors play key roles in both inflammation and cancer by promoting proliferation, angiogenesis, and carcinogenesis and by recruiting immune cells. The extracellular matrix is altered in inflammation and provides structural support to developing tumors. Hypoxia is a common state in cancers and inflamed tissues which causes DNA damage and induces tumorigenic factors. Finally, tissue vasculature is a vital part of its microenvironment, supplying oxygen, nutrients, and growth factors to rapidly dividing cells and providing a mechanism for metastatic spread. This review will discuss the reflexive relationship between cancer and inflammation with particular focus on how by considering the role of inflammation in physiologic processes such as the maintenance of tissue

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homeostasis and repair may provide a logical framework for understanding the connection between the inflammatory response and cancer. The cells and molecules outlined here represent potential targets for the treatment of head and neck cancer.

5.1 Introduction

Head and neck squamous cell carcinoma (HNSCC) originates in the mucosa of 5 major anatomic subsites: the oral cavity, oropharynx, larynx, hypopharynx, and nasopharynx. It is the sixth most common cancer worldwide, with approximately 650,000 new cases reported annually. Aggravating factors are tobacco smoking, alcohol consumption, betel chewing, and human papilloma virus (HPV) infection (Curado and Hashibe 2009). In the United States, there were 49,000 new cases in 2010, with 11,000 deaths (Jemal et al. 2010). Despite the overall decreased incidence of HNSCC in the United States over the past 3 decades, researchers have observed a significant increase in the incidence of squamous cell malignancies of the base of tongue, and tonsil, particularly in young-to-middle-age patients likely due to rising incidence of HPV-associated HNSCC (Shiboski et al. 2005).

Despite treatment advances in multimodality therapy with surgery, radiotherapy, and chemotherapy, 5-year survival is still poor for patients with locoregionally advanced disease (Forastiere et al. 2003; Posner et al. 2007; Vermorken et al. 2007).

The genetic alteration of cells in wide preneoplastic fields (field cancerization) results in locoregional recurrence and second primary cancer. Half of all individuals still die from their disease. The characterization of the mechanisms involved in the metastasis formation and the identification of markers allowing identifying patients with biologically aggressive tumors is of great interest for the effective management of HNSCC patients.

Cigarette smoke (CS) causes considerable morbidity and mortality by inducing cancer, chronic lung and vascular diseases, and oral disease. Despite the well-recognized risks associated with smoking, the habit remains unacceptably prevalent. Several toxins present in CS have immune modulatory effects. CS also contains trace amounts of microbial cell components, including bacterial lipopolysaccharide. These and other CS constituents induce chronic inflammation at mucosal surfaces and modify host responses to exogenous antigens. Mucosal damage from chronic tobacco and alcohol exposure has been well characterized, both in terms of its clinicopathologic course and the underlying molecular derangements responsible for tumor development. Premalignant lesions, including leukoplakia and erythroplakia, progress to invasive carcinomas along a well-described pathologic sequence (Perez-Ordonez et al. 2006).

Molecular events that undergird this process include increasing cytogenic abnormalities, inactivation of tumor suppressor genes, and changes in intracellular signaling pathways that induce cellular immortalization. The effects of CS on immunity are far-reaching and complex; both pro-inflammatory and suppressive effects may be induced. The net effect of CS on immunity depends on many variables, including the dose and

type of tobacco, the route, and chronicity of exposure, and the presence of other factors at the time of immune cell stimulation, such as Toll receptor ligands or other inflammatory mediators. CS impairs innate defenses against pathogens, modulates antigen presentation, and promotes autoimmunity. CS also impairs immunity in the oral cavity and promotes gingival and periodontal disease and oral cancer. The recognition of specific mechanisms by which CS affects host immunity is an important step toward elucidating mechanisms of tobacco-induced disease and may identify novel therapeutic approaches for the management of smoking-related diseases (Lee et al. 2012).

Human papilloma virus-related oropharyngeal carcinoma (HPVOPC) clinically behaves differently than tobacco- and alcohol-induced HNSCC. Inflammation and immunosuppression are likely to also play a critical role in HPVOPC. These patients tend to present at a younger age, and without a history of excessive tobacco or alcohol use. Overall, HPVOPC patients also have better outcomes, with tumors more responsive to both surgical and non-surgical therapies and a lower risk of dying from disease. The relationship of HPVOP to inflammation remains largely unexplored (Chung and Gillison 2009; Ang et al. 2010).

5.2 Inflammatory Signaling Pathways Associated with Head and Neck Cancer

There are several genetic alterations associated with chronic inflammation in HNSCC. Inactivation of tumor suppressor genes through homozygous deletion, point mutations, and epigenetic alterations such as hypermethylation fuels the neoplastic process. For instance, a common genetic alteration in 70–80 % of dysplastic squamous cells and HNSCC tumors is the loss of chromosome 9p21, a region that contains cyclin-dependent kinase inhibitor 2A (CDKN2A), encodes tumor suppressor genes p16 and p14, and is involved in the G1 phase cell-cycle regulation. Loss of 3p, a locus with tumor suppressor phenotype, is another common genetic event seen early in dysplasia (Perez-Ordonez et al. 2006). Inactivation of p16 is a frequent event witnessed in >80 % of tumor specimens. Similarly, loss of heterozygosity of 17p—the region encoding tumor suppressor p53—is extremely common (Reed et al. 1996). It has been found that half of all tumor specimens from patients with HNSCC contain p53 mutations. Notably, disruption of TP53—the genetic locus on 17p giving rise to p53—has been associated with reduced survival after surgical therapy for HNSCC (Poeta et al. 2007).

Telomerase, an enzyme active in germ line cells but normally quiescent in somatic cells, has been shown to be overexpressed in 90 % of HNSCC cells. Telomerase is responsible for maintaining genomic stability by protecting chromosomal ends, especially in rapidly dividing cells; its activity in malignant cells enables evasion of apoptosis and contributes to cellular immortality (McCaul et al. 2002). Altered intracellular signaling also facilitates neoplastic development in HNSCC, including activation of oncogenic pathways downstream of the epidermal growth factor receptor (EGFR) and other molecular pathways.

HPV inactivates the same pathways via direct viral effects. The HPV is a circular, double-stranded DNA virus that encompasses many different subtypes, with HPV-16 and HPV-18 being the most common oncogenic variants in HPVOPC. Using in situ hybridization, HPV-16 DNA has been found in up to 72 % of oropharyngeal cancer specimens where this association remains the highest (D'Souza et al. 2007). On a molecular level, HPV gains access to the intracellular compartment of mucosal squamous cells and integrates into host DNA. The integrated virus subsequently expresses oncoproteins E6 and E7, which act synergistically to target the tumor suppressor genes p53 and pRb for ubiquitin-mediated intracellular degradation, resulting in genomic instability and oncogenic transformation as the normal cell-cycle regulatory points are inactivated (Chung and Gillison 2009).

Experimental tumor model studies show that non-steroidal anti-inflammatory drugs (NSAIDs) impair the growth and development of HNSCC, indicating potential as a chemopreventive agent. Furthermore, regular use of NSAIDs and aspirin has been shown to reduce the risk of other cancers.

Biologically, NSAIDs act as non-specific inhibitors for the pro-inflammatory cyclooxygenase enzymes (COX-1 and COX-2), which are involved in the conversion of arachidonic acid (AA) to prostaglandins (PG). COX-1 is present in most tissues and is involved in the production of PGs required for many normal physiologic functions, while COX-2 is found only in a limited number of cell types and is induced by stimulatory factors implicated with inflammation and many cancers.

Overexpression of COX-2 and PGs have been reported in a variety of cancer sites, including HNSCC, with increased levels reported in both tumor tissue and adjacent epithelium in HNSCC but not normal epithelium. Studies also suggest a correlation between COX-2 expression and head and neck tumor size and prognosis, with higher expression correlating with poorer outcome (Wilson et al. 2011). The downstream actions of PGs, such as increased cell proliferation, cell mobility and invasion, neo-angiogenesis, and the inhibition of apoptosis, are known to play important roles in cancer development. The mechanism by which NSAIDs inhibit tumor development is not clearly understood, although it is thought that they may act through the inhibition of COX-2 and consequently the synthesis of PGs and their pro-cancerous downstream effects (Wilson et al. 2011).

It has been shown that the EGFR and COX-2 have an important role in the biology of HNSCC. Overexpression of COX-2 is associated with a poor prognosis in HNSCC, and COX-2 inhibitors have demonstrated synergy when combined with EGFR inhibitors in preclinical models (Chen et al. 2004; Chung et al. 2011). Inflammatory mediators can promote epithelial–mesenchymal transition (EMT), a process by which epithelial cells lose their cell polarity and cell–cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. This process is responsible for the increase resistance to EGFR-TKIs in HNSCC. These studies provide a strong rationale for combining a COX-2 inhibitor with an EGFR TKI (Kao et al. 2011).

Recent advances in the understanding of the oncogenesis of HNSCC have revealed multiple deregulated signaling pathways. Transforming growth factor-β (TGF-β) and PTEN/PI3K/Akt/mTOR pathways are among the most frequently altered signaling routes. Both pathways have central roles in numerous cellular

processes, including metabolism, cell growth, apoptosis, survival, and differentiation, which ultimately contribute to HNSCC progression (Molinolo et al. 2009).

NF-κB is a pleiotropic transcription factor which plays a role in both innate and adaptive immunity and is required for the expression of several pro-inflammatory factors. Chronic inflammation is often a key factor in cancer development. As the head and neck area is prone to exposure to factors causing irritation and inflammation of the squamous epithelium, it might therefore be plausible that chronic inflammation also might be a major cause for the development of HNSCC. It has been shown that NF-κB and its pro-inflammatory target genes are activated in HNSCC cell lines and tumor specimens. Blocking NF-κB function in HNSCC greatly reduces tumor growth and decreases the expression of IL-6 and IL-8 along with many other cytokines and chemokines associated with the pro-inflammatory state (Kross et al. 2010).

Cytokines are soluble proteins that play an important role in the initiation and maintenance of inflammatory and immune responses as well as intercellular cross talking. Cytokines regulate immunity, inflammation, and hematopoiesis, and this family of proteins includes interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs), and growth factors. They are typically divided into two categories: pro-inflammatory (e.g., IL-1, IL-6, IL-8, TNF-α, and IFN-γ) and anti-inflammatory [e.g., IL-4, IL-10, TGF-β, and vascular endothelial growth factor (VEGF)]. They bind to receptors and transducer signals via second messengers to control growth, differentiation, and activation of cells (Wang et al. 2009). It has been shown that high levels of cytokines and growth factors may have a role in the development of different cancers (Wang et al. 2009). High levels of IL-1a, IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF), growth-regulated oncogene-(a) GRO1, VEGF, and hepatocyte gowth factor (HGF) have been involved in the development of HNSCC (Lee et al. 2007). It is also important to notice that altered levels of cytokines and growth factors can predict response to therapy and high levels of pro-inflammatory cytokines are associated with poor outcomes in patients undergoing chemoradiation treatments for HNSCC (Allen et al. 2007).

Interleukin-6 (IL-6) is a multifunctional cytokine synthesized in response to stimuli such as infection and trauma by a variety of cells such as macrophages, neutrophils, keratinocytes, fibroblasts, and endothelial cells. IL-6 cell signals are transmitted through a receptor expressed in a wide range of target cell types. In addition to this, a soluble IL-6 receptor (sIL-6R) enables to widen the repertoire of cells responsive to IL-6 (Jones et al. 2001). IL-6 is able to stimulate a number of biologic processes including antibody (and probable autoantibody) production, activation of T cells, B cell differentiation, increase in acute-phase proteins, hematopoiesis, induction of angiogenesis, vascular permeability, and osteoclast differentiation (Ridker et al. 1997; Nibali et al. 2012). It is also a strong stimulator of hepcidin, a liver-produced hormone that regulates intestinal iron absorption (Hohaus et al. 2010), potentially contributing to sideropenic anemia in chronic inflammation. IL-6 activity in inflammation is considered double-edged, acting both as anti-inflammatory (e.g., down-regulation of neutrophil

recruitment and pro-inflammatory cytokine expression) (Xing et al. 1998) but also as pro-inflammatory (e.g., induction of acute-phase reactants by the liver) in chronic diseases (Jones et al. 2001). IL-6 is also believed to have growth factor properties regarding the development and progression of many types of cancers (Nishimoto 2010).

5.3 Role of Inflammatory Molecules in the Invasion, Metastasis and Angiogenesis of Head and Neck Cancer Cells

Epidemiologic and experimental evidence supports the concept that chronic inflammation promotes the development and progression of cancers. Because inflammation is a complex process involving many effector cells and mediators, it is likely that inflammation facilitates tumor progression through multiple mechanisms (Balkwill and Mantovani 2001).

The initiation of an epithelial-to-mesenchymal transition (EMT) is required for tumor dissemination to occur. E-cadherin has a key role in epithelial intercellular adhesion and its down-regulation is a hallmark of EMT, which is associated with invasion, metastasis, and poor prognosis. EMT is the major mechanism responsible for mediating invasiveness and metastasis of epithelial cancers. E-cadherin transcriptional repressors have a role in the inflammation-induced promotion of EMT in HNSCC, which is mediated by COX-2. Levels of COX-2 and its catalytic product PGE2 are increased in HNSCC (Buchanan et al. 2003; Dannenberg and Subbaramaiah 2003; Cooper et al. 2004). PGE2 can stimulate cell proliferation, motility, and angiogenesis while inhibiting apoptosis and immune surveillance (Buchanan et al. 2003; Cooper et al. 2004). COX-2-derived PGE2 may also promote metastasis by stimulating EMT and cell invasion (Dohadwala et al. 2006). It has been reported that PGE2 is transported or passed through the cell membrane via pro-staglandin-specific transporters, including the pro-staglandin transporter (PGT, an influx transporter). Intratumoral PGE2 levels depend not only upon the rate of production, but also on the rate of degradation. Inactivation of PGE2 located in the developing tumor microenvironment has been suggested to occur by a two-step model (Haddad et al. 2009). The first step is mediated by the PGT, which engages carrier-mediated membrane transport of pro-staglandins, including PGE2, PGF2a, and PGD2, from the extracellular milieu to the cytoplasm (Haddad et al. 2009). This transporter belongs to the organic anion superfamily of transporting polypeptides that contain 12 transmembrane spanning domains. The second step of PGE2 inactivation occurs in the cytoplasm, where 15-hydroxyprostaglandin dehydrogenase (15-PGDH) catabolizes and thus inactivates PGE2 (Haddad et al. 2009). Studies have shown that 15-PGDH expression is frequently reduced in several other epithelial cancers as well, (Ichikawa et al. 1996; Holla et al. 2008) suggesting that abnormalities in catabolism of PGE2 may have an important role in the development of these cancers.

During the process of tumor dissemination, tumor cells lose their epithelial characteristics [inhibition of E-cadherin (Cdh1)] to the profit of mesenchymal properties (expression of Snail1 and increased migratory abilities), allowing them to invade blood and lymphatic systems and establish new colonies in distant organs (Thiery et al. 2009). Other inflammatory mediators in addition to COX-2 have been shown to modulate EMT. Indeed in HNSCC cell lines, IL-1β was reported to stimulate Snail1 and inhibit Cdh1 expression (John et al. 2009). Among inflammatory actors, IL-32 was reported to modulate cytokine expression and to be up-regulated by TNF-α, IL-1β and IL-6 (Shioya et al. 2007; Kim et al. 2005). IL-8 and GRO1 serve as chemoattractants for neutrophils, monocytes, and endothelial cells, which are all major constituents of the inflammatory and angiogenesis response, and their expression promotes aggressive growth and metastasis (Van Waes 2007). In addition, IL-1 and IL-6 are potent inducers of HGF production by stromal cells, such as fibroblasts, further enhancing IL-8 and VEGF expression (Worden et al. 2005). Several cytokines and growth factors also activate signal pathways that promote the malignant phenotype. TNF-α, IL-1, HGF, and their receptors promote activation of the mitogen-activated protein kinase-activator protein-1 (MAPK-AP-1), nuclear factor-kappa B (NF-κB), and phosphatidylinositol-3 kinase (PI3K)/Akt pathways (Van Waes 2007). Epidermal growth factor (EGF) and IL-6 activate signal transducer and activating transcription factor-3 (STAT3) in HNSCC cells (Lee et al. 2008).

An increasing number of studies have recently focused on the role of cytokine networks, including IL-6, in the pathogenesis and progression of oral malignancy. In particular, clinical studies reported elevation of IL-6 levels in serum and saliva of patients with oral and other cancers of the head and neck compared with age-matched control subjects and their significant relation with staging and response to therapy (Chen et al. 1999; Bigbee et al. 2007). The expression of IL-6 and IL-8 genes was shown, via large-scale gene expression profiling on laser-captured microdissected oral cancer and normal oral epithelial cells, to be uniquely associated with HNSCC (Alevizos et al. 2001). IL-6 seems to contribute to oral cancer pathogenesis through different mechanisms and biologic processes. An in vitro study showed that IL-6 can stimulate HNSCC cells to enhanced secretion of matrix metalloproteinases 1 and 9, which play a major role in infiltrative growth, metastasis, and neo-angiogenesis (Sundelin et al. 2005). IL-6 may also modulate a variety of keratinocytes pathways including cell growth, survival, and differentiation. In particular, IL-6 has been shown to stimulate proliferation of cultured human keratinocytes in psoriatic skin (Nibali et al. 2012). Furthermore, IL-6 can activate transcription factors such as signal transducer and activator of transcription (STAT)-1 and STAT-3, which in turn have been observed in various tumors (Hirano et al. 2000). A recent study showed that IL-6 can also promote tumorigenesis by causing DNA hypomethylation as well as aberrant promoter hypermethylation changes, which can lead to epigenetic changes in gene expression of HNSCC cells (Gasche et al. 2011). Furthermore, in vitro studies demonstrated that oral keratinocytes can produce IL-6 in response to a number of environmental factors

well known to increase oral cancer risk such as areca nut and tobacco smoking (Jeng et al. 2003). Indeed, biopsies from individuals with oral submucous fibrosis showed increased expression of IL-6 in the epithelium and underlying inflammatory infiltrate, as well as in peripheral blood mononuclear cells (Haque et al. 2000).

IL-32 is one of the cytokines with pro-inflammatory activities implicated in inflammatory disorders, such as rheumatoid arthritis, mycobacterium tuberculosis infections, and inflammatory bowel disease (Shioya et al. 2007; Heinhuis et al. 2011). On a retrospective study of 65 patients with HNSCC, it was shown that patients with tumors expressing high amounts of IL32 had a worse disease-free survival and overall survival in comparison with individuals with weak IL32 tumor expression. In addition, in vitro data linked IL32 expression to metastatic potential (Guenin et al. 2013). The inverse correlation between IL32 and p53 expression found in this study was also found in patients with hepatocarcinoma (Kang et al. 2012). The increased p53 expression induced by IL-32 inhibition could originate from the loss of Snail1 which would not be able to form a complex with p53 leading to its degradation through a transcription-independent mechanism (Lee et al. 2009). Alternatively, IL-32 inhibition was reported to decrease NF-κB which is a well-described p53 inhibitor and an activator of Snail1 expression (Gurova et al. 2005; Tergaonkar and Perkins 2007; Zhang et al. 2011). Therefore, IL-32 down-regulation might allow p53 re-expression through NF-κB and Snail1 inhibition (Kim et al. 2011). We can speculate that IL32 plays a pivotal role in tumor responses to inflammatory mediators and enhances cell invasiveness properties through a nuclear NF-κB/Snail1 axis in which intermediary actors have to be identified. This is supported by its nuclear localization found in the more aggressive tumors (Guenin et al. 2013).

TGF-β belongs to a superfamily of multifunctional cytokines that regulate cell proliferation, differentiation, migration, adhesion, and apoptosis, thereby influencing important physiologic processes such as embryonic development, immune function, and carcinogenesis (Derynck and Zhang 2003; Massague 2008). The three mammalian TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3, exert their functions through a cell-surface receptor complex composed of type I (TGFBR1) and type II (TGFBR2) serine/threonine kinase receptors. Upon ligand binding, TGFBR2 recruits and phosphorylates TGFBR1, which in turn phosphorylates Smad2 or Smad3. Phosphorylated Smad2 or Smad3 binds to Smad4, and then, these complexes translocate from the cytoplasm into the nucleus. This results in the transcriptional activation of TGF-β-responsive genes that mediate the effects of TGF-β at the cellular level. In addition to Smad-mediated signaling, receptor activation also induces other downstream targets, including Ras, RhoA, TAK1 (TGF-β-activated kinase-1), MEKK1, PI3K, and PP2A, to produce the full spectrum of TGF-β response (Moustakas and Heldin 2009; Zhang 2009).

The effects of TGF-β signaling on carcinogenesis largely depend on the tissue of origin and the tumor type. In most types of human cancer, TGF-β has a paradoxical role in cancer development by way of functioning as a tumor suppressor during the early stages (Engle et al. 1999) and as a tumor promoter during the later stages (Piek and Roberts 2001; Tang et al. 2003). Several reports have noted

that mutations and polymorphisms of TGFBR1 and Smads are associated with HNSCC, (Chen et al. 2001; Xie et al. 2003; Pasche et al. 2005) suggesting that TGF-β functions as a potent tumor suppressor. However, it is not clear whether alterations in TGF-β signaling act alone or in concert with alterations in other pathways to promote a pro-oncogenic phenotype in advanced late-stage HNSCC.

As noted above, The PI3K/Akt pathway is important for suppressing apoptosis, and promoting cell growth and proliferation. In HNSCC, hyper activation of PI3K can be induced by mutations or by enhanced activity of its upstream activators, including the activation of Ras oncoproteins or inactivation of phosphatase and tensin homolog (PTEN) deleted on chromosome 10 (Molinolo et al. 2009). PTEN is a potent tumor suppressor gene and a negative regulator of the PI3K/Akt pathway. As PTEN mutations were identified in 0–16 % of HNSCCs, loss of PTEN expression was observed in 29 % of tongue cancers and loss of heterozygosity of the PTEN locus was identified in 40 % of HNSCCs (Henderson et al. 1998; Shao et al. 1998; Lee et al. 2001). Additionally, 47 % of HNSCC cases showed at least one molecular alteration in the PI3K/Akt pathway, including PI3 KCA and AKT2 amplification, p110α overexpression and PTEN protein down-regulation. This suggests the critical role of the PTEN/PI3K/Akt signaling pathways in the carcinogenesis of HNSCC (Pedrero et al. 2005). It seems that there may be negative cross talk between the TGF-β tumor suppressor and the PI3K/Akt pathways (Bian et al. 2012). It was shown that defects in the TGF-β and PI3K/Akt signaling pathways are common in human HNSCCs. Activation of the PI3K/Akt pathway due to PTEN deletion initiates tumor formation by increasing proliferation in the head and neck epithelia. However, PTEN deletion alone is not sufficient to induce invasive HNSCC due to the induction of premature senescence by p-Akt in the presence of the tumor suppressor TGF-β. In combination with the additional loss of TGFBR1, which blocks tumor inhibition by TGF-β signaling, premalignant cells cannot undergo cellular senescence and will progress into cancer cells (Bian et al. 2012).

Studies on a 2cKO mouse model showed that TGFBR1 and PTEN work collaboratively in suppressing tumor progression. The loss of TGFBR1/PTEN function is associated with increased cell proliferation, loss of apoptosis, and increase levels of Cyclin D1 (CCND1) in head and neck cancer (Bian et al. 2012).

The multifunctional cytokine TGF-B has different effects in premalignant and malignant cells. In epithelial cells, TGF-B has a tumor-suppressor effect via its autocrine interaction with other signaling pathways. On the other hand, in tumor cells, TGF-B increases tumor proliferation via its paracrine effects which include but not limited to inflammation, angiogenesis, and escape form immunosurveillance (De Wever and Mareel 2003).

The interaction between different pathways, transcription factors, and multifuncitonal cytokines is far more complex than previously thought. For instance, in a head and neck mouse model, it was recently shown that the deletion of TGFBR1/PTEN is associated with the activation of the NF-kB pathway. As a result of this interaction, several genes that are associated with an inflammatory state are also over-expressed (i.e., Cxc11, Cxcl5, Ptgs2). This pro-inflammatory state is responsible for the recruitment of myeloid-derived suppressor cells

(MDSCs), which increases the angiogenesis and immune suppressive state within the tumor stroma (Bian et al. 2012). The disruption of the TGF-B signaling pathway can lead to similar findings (Lu et al. 2006; Bierie et al. 2008). These data support the concept that the tumor stroma has a pivotal role in the development and progression of head and neck cancer (Bian et al. 2012).

Neuroblast differentiation-associated protein AHNAK, also known as desmoyokin, is a protein that in humans is encoded by the AHNAK gene. AHNAK was originally identified in 1989 (in bovine muzzle epidermal cells) and named desmoyokin due to its localization pattern (that resembled a yoke) in the desmosomal plaque. It is a protein of exceptionally large size (700 kDa) that is expressed in a variety of cell types (Shtivelman et al. 1992). This protein has the ability to shuttle between various subcellular compartments. For instance, it has been shown that AHNAK can translocate from the cytoplasm to the plasma membrane of keratinocytes in a manner dependent on Ca^{2+} and protein kinase C (Hashimoto et al. 1995). Furthermore, AHNAK was shown to contain a nuclear export signal (NES) sequence which allowed it to be excluded from the nuclei of epithelial cells following cell–cell contact and activation of protein kinase B, respectively (Sussman et al. 2001). At functional level, AHNAK was shown to be involved in various cellular processes, including calcium regulation and organization of the actin cytoskeleton (Haase et al. 1999; Gentil et al. 2001). In tumor cells, AHNAK was recently found to be essential for pseudopodia formation and tumoral migration/invasion (Shankar et al. 2010). Other recent studies proposed that the AHNAK gene might be involved in mutagenic transformation of colon epithelial cells and thus carcinogenesis (Tanaka et al. 2008). It is well established that solid tumors display an inflammatory microenvironment characterized by large numbers of tumor-infiltrating immune cells (Coussens and Werb 2002). Within this microenvironment, the immune cells of the host are reprogrammed by the tumor cells to acquire pro-tumoral activities. Although less characterized than tumor-associated macrophages (TAMs) or tumor-infiltrating lymphocytes (TILs), tumor-infiltrating neutrophils are emerging as important players in the pathophysiology of cancer. Within the tumor tissue, neutrophils can modulate several cellular processes which may ultimately lead to tumor progression. Neutrophils were shown to modulate angiogenesis in several murine tumor models (Nozawa et al. 2006; Jablonska et al. 2010; Bekes et al. 2011) and were recently associated with angiogenesis progression in hepatocellular carcinoma patients (Kuang et al. 2011). Further studies showed that neutrophils could directly modulate the biology and functions of tumor cells by promoting their migration, invasion or proliferation (Gregory and Houghton 2011).

There is an association of high numbers of tumor-infiltrating neutrophils with advanced disease and poor clinical outcome in patients with different types of cancer, such as renal cancer, hepatocellular cancer, non-small-cell lung carcinoma (NSCLC), or melanoma (Dumitru et al. 2012).

In head and neck cancer patients, it was demonstrated that a high neutrophilic infiltration of the tumor tissue was correlated with high tumor stage and poor survival (Trellakis et al. 2011). In vitro studies indicated a direct interaction between

neutrophils and head and neck cancer cells by showing that neutrophils were primed by the tumor cells to release pro-inflammatory factors, which promoted tumoral migration in a feedback manner (Dumitru et al. 2011, 2012). Selected soluble inflammatory mediators, such as cytokines, chemokines, and metabolites of the AA pathway, have been found to change the function and differentiation of immune cells (Lin and Karin 2007). Among these molecules, macrophage migration inhibitory factor (MIF) is emerging as an important regulator of inflammation in cancer (Bucala and Donnelly 2007). A number of studies found that high levels of MIF in the tumor tissues or serum of patients with different types of cancer were associated with advanced disease and poor clinical outcome (Grieb et al. 2010). It was also demonstrated that overexpression of tumoral MIF was associated with poor overall survival in patients with orohypopharyngeal cancer (Dumitru et al. 2011). More importantly, MIF was identified as one of the missing links in the tumor-neutrophil interaction and showed that head and neck cancer cells released MIF which subsequently enhanced the pro-inflammatory functions of neutrophils to promote tumoral migration (Dumitru et al. 2011). AHNAK overexpression is associated with poor survival in these patients. Interestingly, in patients with HNSCC, it was found that high levels of AHNAK together with high MIF expression or high neutrophilic infiltration, respectively, were strongly associated with poor survival. Synchronous high levels of MIF and tumor-infiltrating neutrophils had stronger predictor values over the individual markers as well. Finally, patients with high levels of all three markers displayed the shortest survival in the entire patient cohort (Dumitru et al. 2013). These findings suggest that AHNAK might cooperate with MIF and/or neutrophils to enhance progression of HNSCC. There is data regarding direct interactions between HNSCC-derived MIF and neutrophils both in vitro and in vivo. It was shown that HNSCC-derived MIF enhanced neutrophil chemotaxis in vitro and that tumoral MIF levels correlated with the neutrophilic infiltration in tissues from orohypopharyngeal carcinoma patients (Dumitru et al. 2011). Since MIF is a known ligand for CXCR2, one of the major chemokine receptors on neutrophils, (Bernhagen et al. 2007) MIF-mediated recruitment might be a critical mechanism for infiltration of HNC tissues by neutrophils. It was further demonstrated that HNC-derived MIF stimulated neutrophils to release large amounts of pro-inflammatory factors, among which CCL4 and MMP9 (Dumitru et al. 2011). Neutrophils enhance the motility, migration, and invasion of tumor cells via—not fully identified—soluble factors and molecular mechanisms (Dumitru et al. 2012). Interestingly, AHNAK was recently linked to regulation of tumoral migration/invasion. It seems that AHNAK is essential for rearrangement of the actin cytoskeleton and pseudopodia formation (Shankar et al. 2010).

HPV-HNSCC differs from tobacco-related head and neck cancers in several ways. The patients tend to be younger in age, lack a significant tobacco and/or alcohol history, and have improved clinical outcomes. The virus-related tumors arise from the deep crypts within the lymphoid tissue of the tonsil and base of tongue and the majority can be distinguished from tobacco-related HNSCC by the characteristic infiltration of lymphocytes in the stroma and tumor nests. Nevertheless, despite this profound inflammatory response, HPV-HNSCCs are able to evade immune surveillance, persist, and grow (Gillison et al. 2008).

Various mechanisms have been proposed for the resistance of human solid tumors to immune recognition and obliteration, including the recruitment of regulatory T cells, MDSCs, and local secretion of inhibitory cytokines. Recent evidence suggests that tumors develop physiologic mechanisms of tissue protection from inflammatory destruction via up-regulation of immune inhibitory ligands. Antigen-induced activation and proliferation of T cells are regulated by the temporal expression of both co-stimulatory and co-inhibitory receptors and their cognate ligands (Topalian et al. 2012).

In the context of cancer, in which immune responses are directed against antigens specifically or selectively expressed by tumor cells, these immune checkpoints can represent major obstacles to the generation and maintenance of clinically meaningful anti-tumor immunity. CTLA-4 and programmed cell death-1 (PD-1) are two such checkpoint receptors being actively targeted in the clinic (Lyford-Pike et al. 2013).

It has been shown that in HPV-HNSCCs that are highly infiltrated with lymphocytes, PD-L1 expression on both tumor cells and CD68(+) TAMs is geographically localized to sites of lymphocyte fronts, whereas the majority of CD8þ TILs express high levels of PD-1, the inhibitory PD-L1 receptor. Significant levels of mRNA for IFN-γ, a major cytokine inducer of PD-L1 expression, were found in HPV(+) PD-L1(+) tumors. These findings support the role of the PD-1: PD-L1 interaction in creating an "immune-privileged" site for initial viral infection and subsequent adaptive immune resistance once tumors are established and suggest a rationale for therapeutic blockade of this pathway (Lyford-Pike et al. 2013).

5.4 Role of Inflammatory Molecules in the Development of Head and Neck Cancer: Evidence from In Vivo Studies

Chronic inflammation is frequently associated with malignant growth and is thought to promote and enhance tumor progression, although the mechanisms which regulate this relationship remain elusive (Coussens and Werb 2002). It has been reported that interleukin (IL)-1β promoted tumor progression by enhancing the accumulation of MDSCs and hypothesized that inflammation leads to cancer through the production of MDSCs which inhibit tumor immunity (Bunt et al. 2006) if inflammation-induced MDSCs promote tumor progression by blocking anti-tumor immunity, then a reduction in inflammation should reduce MDSC levels and delay tumor progression; whereas an increase in inflammation should increase MDSC levels and hasten tumor progression (Dinarello 1996). This hypothesis was tested by using the 4T1 mammary carcinoma and IL-1 receptor (IL-1R)–deficient mice which have a reduced potential for inflammation, and IL-1R antagonist–deficient mice, which have an increased potential for inflammation. Consistent with the initial hypothesis, IL-1R–deficient mice have a delayed accumulation of MDSC and reduced primary and metastatic tumor progression. Accumulation of MDSCs and tumor progression are partially restored by IL-6, indicating that IL-6

is a downstream mediator of the IL-1B-induced expansion of MDSC. In contrast, excessive inflammation in IL-1R antagonist-deficient mice promotes the accumulation of MDSC and produces MDSC with enhanced suppressive activity. These results show that immune suppression by MDSC and tumor growth are regulated by the inflammatory milieu and support the hypothesis that the induction of suppressor cells which down-regulate tumor immunity is one of the mechanisms linking inflammation and cancer (Bunt et al. 2007).

The potential role of TGFBR1/PTEN in development of head and neck cancer was studied in the 2cKO mouse model. It was found that deletions of TGFBR1/PTEN are associated with tumor cells with a proliferative and invasive phenotype. Interestingly, the nonmalignant epithelial cells of the head and neck area also revealed and enhanced proliferation pattern, loss of apoptosis, and increased expression of CCND1 (Bian et al. 2012). The effects of TGF-B was shown to have different effects on premalignant and malignant cells. On premalignant cells, TGF-B exerts tumor-suppression effects through its autocrine interaction with other signaling pathways. The effect of TGF-B on tumor cells is exert by its paracrine activity and is associated with an aggressive tumor phenotype and a pro-inflammatory state (De Wever and Mareel 2003). There is increasing evidence that the tumor micro-environment has an important role in cancer development and tumor progression. For instance, deletions of TGFBR/PTEN in the mouse head and epithelium are associated with activation of the NF-kB pathway, the generation of a pro-inflammatory stroma. As a result of all these events, there is a recruitment of MDSC's and increase angiogenesis, and an immuno-suppressive state of the tumor micro-environment that facilitates the proliferative and infiltrating pattern of head and neck tumor cells (Bian et al. 2012; Lu et al. 2006; Bierie et al. 2008)

5.5 Evidence from Patients for the Role of Inflammation in Head and Neck Cancer

It is well established that high levels of pro-inflammatory cytokines play a role in the development of HNSCC (Wang et al 2009). In clinical practice, it has been shown that low levels of cytokines and growth factors are associated with response to therapy and high levels are associated with poor outcomes in patients with HNSCC receiving chemotherapy and radiation. (Allen et al. 2007).

It has been shown that there is a significant reduction in HNC risk with aspirin use, with the strongest protective effect for laryngeal cancers. A subanalysis in individuals with information on alcohol use revealed an increasing reduction in HNC risk, albeit non-significant, with aspirin use among participants with increasing alcohol use. The exact mechanism by which this may be occurring is uncertain. Ethanol found in alcohol has been reported to act as a local irritant potentially leading to localized inflammation, which may possibly explain the observed reduction in HNC in aspirin users who consume alcohol.

In patients with HPV-related oropharyngeal cancer, there is some evidence to suggest an up-regulation of COX-2 in HPV-infected tissues, and this might explain the reduction in HNSCC in this patient population (Wilson et al. 2013). However, the chemopreventive effect of aspirin and NSAIDs cannot be explained by the inhibition of pro-staglandin synthesis alone, since several NSAIDs have anti-proliferative effects in cells without COX activity. High aspirin doses induce apoptosis through COX-independent mechanisms, by regulating several different targets—e.g., ALOX15, a pro-apoptotic gene PAWR, and an anti-apoptotic gene BCL2L1. Additionally, NSAIDs including aspirin also induce apoptosis by the activation of caspases, the activation of p38 MAP kinase, release of mitochondrial cytochrome c, and activation of the ceramide pathway. These effects might not be universal to all cell types and the range of doses of aspirin needed in such COX-independent pathways could be higher than for the inhibition of COX-2 (Elwood et al. 2009). Celecoxib, in conjunction with erlotinib and reirradiation, was shown to be a feasible and clinically active regimen in a population of patients with recurrent HNSCC who had a poor prognosis (Kao et al. 2011). However, the majority of data suggest a limited role for celecoxib in head and neck cancer therapy, either due to toxicity or lack of efficacy (Dannenberg and Subbaramaiah 2003; Jaeckel et al. 2001). Celecoxib was ineffective in controlling oral premalignant lesions in a recent randomized controlled trial (Papadimitrakopoulou et al. 2008). COX-2 inhibition has a chemopreventive effect, but its application as a treatment of HNSCC in a clinical setting still requires further research to overcome its limited anticancer effects (Kim et al. 2010).

Apricoxib is a selective COX-2 inhibitor with preclinical data showing analgesic, anti-inflammatory, and anti-tumor effects. Apricoxib plus erlotinib was tested in a phase I study in non-small-cell lung cancer and was found to be well tolerated with a 60 % disease control rate (Reckamp et al. 2011). In addition to reversing EMT via inhibition of COX-2, Apricoxib up-regulates 15-prostaglandin dehydrogenase and the PGT, thereby reducing the levels of active PGE2 by both suppressing its synthesis and increasing its catabolism (St John et al. 2012). Treatment of HNSCC cells with Apricoxib also causes greater up-regulation of E-cadherin expression and down-regulation of vimentin, as compared to celecoxib treatment. This has significant implications for targeted chemoprevention and anticancer therapy because E-cadherin expression has been implicated as a marker of sensitivity to EGFR TKI (St John et al. 2012). Studies have shown that EGFR and COX-2 have an important role in the biology of HNSCC. Overexpression of COX-2 is associated with a poor prognosis in HNSCC, and COX-2 inhibitors have demonstrated synergy when combined with EGFR inhibitors in preclinical models (Chen et al. 2004; Chung et al. 2011). Inflammatory mediators can promote EMT and increase resistance to EGFR-TKIs in HNSCC. These studies provide a strong rationale for combining a COX-2 inhibitor with an EGFR TKI.

In patients with HPVOPC, the PD-1: PD-L1 pathway plays a role in both persistence of HPV infection (through expression of PDL1in the tonsillar crypt epithelium—the site of initial infection) as well as resistance to immune elimination during malignant progression. Given the high levels of membranous PD-L1 expression within the tumors, recent studies support a rationale for administering PD-1/PD-L1-targeted therapy to the HPVOPC patient population (Topalian et al. 2012).

5.6 Conclusions and Future Directions

In conclusion, current evidence supports the concept that chronic inflammation promotes the development and progression of cancer. Because inflammation is a complex process involving many effector cells and mediators, it is likely that inflammation facilitates tumor progression through multiple mechanisms that are not yet fully understood. Tumor promotion and progression are dependent on physiologic responses provided by supportive tissues of the tumor environment but that are not necessarily cancerous themselves. Infiltration of immune cells facilitates tumor development through production of factors that promote carcinogenesis and by enabling tumors to evade the host immune response. Small molecules including cytokines, chemokines, and growth factors play key roles in both inflammation and cancer by promoting proliferation, angiogenesis, and carcinogenesis and by recruiting immune cells. Many of these physiologic processes and small molecules are potential targets with anti-neoplastic activity.

From in vitro and in vivo data, it seems that in the future, the use of different molecules that can affect one of the inflammatory pathways at different levels (i.e., co-administration of EGFR and STAT inhibitors) or different pathways at different levels (i.e., COX-2 inhibitors, NF-κB, and STAT inhibitors) will probably be needed to improve the anti-neoplastic activity of these molecules.

The role of the PD-1: PD-L1 interaction in creating an "immune-privileged" site for initial viral infection and subsequent adaptive immune resistance once tumors are established supports the rationale for therapeutic blockade of this pathway in patients with HPVOPC.

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