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Current understanding of the tumor microenvironment of laryngeal dysplasia and progression to invasive cancer

Sumita Trivedi^a, Clark A. Rosen^a, and Robert L. Ferris^{a,b,c,d}

^aDepartment of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, USA

^bDepartment of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, USA

^cCancer Immunology Program, University of Pittsburgh Cancer Institute, Pittsburgh, USA

^dTumor Microenvironment Center, University of Pittsburgh Cancer Institute, Pittsburgh, USA

Abstract

Purpose of review—This review examines the historical tumor progression genetic model of laryngeal carcinomas, from dysplasia to invasive carcinoma and the role of infiltrating immune and inflammatory cells as contributors to this process.

Recent findings—Classically, the genetic model of carcinogenesis describes overexpression of oncogenes and/or silencing of tumor suppressor genes which, when combined with exposure to environmental carcinogens over the course of time, results in damage to cellular DNA. Increasing evidence indicates that innate and adaptive immune mediators also play an important role in tumor progression of laryngeal carcinomas. Cellular mediators of immune suppression are often over represented in the tumor microenvironment and these cells release cytokines, which perpetuate immune suppression allowing for tumor immune evasion.

Summary—Future therapies targeting laryngeal malignancies should focus on a combined approach which targets both genetic variations and immune mediators.

Keywords

dysplasia; genetic mutations; immune mediators; laryngeal carcinoma

INTRODUCTION

Although there has been a steady decrease in the incidence of laryngeal cancers in the United States, it remains a significant cause of global morbidity and mortality [1]. As with all cancers of the head and neck, clinical stage at presentation is the single most important factor influencing overall prognosis, suggesting that early diagnosis is crucial to improve clinical outcomes [2]. Premalignant lesions of the larynx are defined as dysplastic epithelial

Correspondence to Robert L. Ferris, MD, PhD, 5117 Centre Ave, Suite 2.26b, Pittsburgh, PA 15213, USA. Tel: +001 412 647 4654; ferrrl@upmc.edu.

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lesions from low grade to high grade that may progress to carcinoma *in situ* [3]. Laryngeal dysplasia occurs in 2–10/100 000 of the population [4] with a 14% rate of malignant transformation [5]. Although the progression from laryngeal dysplasia to carcinogenesis remains incompletely understood, it is believed to be a stepwise process, where genetic changes are followed by architectural and cytological changes in the squamous epithelium [6]. The tumor, its surrounding stroma and cellular infiltrates form the tumor microenvironment. Immune cells, both of the innate and adaptive systems, as well as other cytokines and signaling molecules, contribute to this tumor microenvironment. Although some immune cells possess antitumoral properties, others function to suppress antitumor immunity and are manipulated by the tumor to evade the immune system. As with other head and neck cancers (HNCs), increasing evidence suggests that innate and adaptive immune mediators in the tumor microenvironment play an important role in tumor progression of laryngeal carcinomas [7]. The prevailing belief that dysplastic progression to malignancy is a result of acquired genetic alterations may only be part of the larger picture. Increasing evidence suggests that host responses can determine variability between dysplastic lesions that progress to malignancy and those that do not [8[■]].

KEY POINTS

- The historical tumor progression model which describes overexpression of oncogenes and/or silencing of tumor suppressor genes is partly responsible for the progression of dysplastic lesions of the larynx to invasive carcinoma.
- Host responses can contribute to the variability between patients who progress to invasive carcinoma.
- Cellular components of the innate and adaptive immune system can promote tumor immune evasion both directly and as a result of cytokine secretion.
- A combined approach targeting both neoantigens and genetic variations in the tumor microenvironment may guide future therapeutic interventions.

RISK FACTORS

Tobacco smoking has long been established as a risk factor for the development of laryngeal malignancy [9–11]. The role of excessive alcohol consumption in the development of laryngeal malignancies remains unclear. Experimental studies indicate that alcohol itself is not a carcinogen, but may promote carcinogenesis through alternate mechanisms, such as enhancing the effects of tobacco and other environmental carcinogens [12,13]. It has been suggested that a possible association between gastro-esophageal reflux disease and laryngeal dysplasia and malignancy may exist, however, a causal link is yet to be demonstrated [14]. Unlike other HNCs, a role for human papillomavirus (HPV) infection in the development of laryngeal cancers is not clearly established but may comprise 5% of cases. A recent meta-

analysis conducted by Li *et al.* [15] found a significant association between HPV infection and laryngeal cancer risk, with the greatest prevalence of HPV type 16 and 18.

HISTORICAL TUMOR PROGRESSION MODEL

The basis of the historical model of tumor progression describes the overexpression of oncogenes and/or silencing of tumor suppressor genes. The accumulation of these changes combined with lifetime exposure to environmental carcinogens such as smoking results in significant DNA damage. In epithelial cells, it is believed that DNA damage causes dysplastic changes which progresses stepwise from mild to moderate to severe, then to carcinoma *in situ*, with subsequent development of invasive cancer. Indeed, cancers of the head and neck are known to be among the most highly mutated malignancies [16]. Some of the most commonly overexpressed and mutated genes in HNC include the epidermal growth factor receptor (EGFR), p53, and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) [17]. Arguably the most commonly studied oncogene in HNC is EGFR as it is overexpressed in 80–90% of cases. EGFR has been found to be overexpressed in both laryngeal dysplasia and invasive malignancies [18–20]. As a regulator of cell growth, migration, and survival, EGFR overexpression confers significant oncogenic potential. Shin *et al.* [21] found that EGFR overexpression dramatically increased in the progression from laryngeal dysplasia to carcinoma. Anti-EGFR monoclonal antibodies have been Food and Drug Administration approved for clinical use and although not effective in a large subset of patients, they remain an important treatment modality alone or in combination with chemoradiotherapy. Another significant oncogene found to be commonly mutated in HNC is PIK3CA. This gene is important in the PI3K signaling pathway, which directly affects tumor cell growth, metabolism, and survival [22]. Approximately, 6–21% of HNC are found to have PIK3CA mutations, representing an important target for therapy. Indeed, clinical trials with inhibitors of PI3K and its associated downstream molecules are currently underway [23,24].

p53, one of the first tumor suppressor genes to be found in many tumor types, is a gene involved in apoptosis and cell cycle regulation [25]. Loss-of-function p53 mutations have been found commonly in both laryngeal dysplasias and carcinomas [26]. Murine models of HNC demonstrate that knockout of the p53 gene results in metastatic carcinomas in 100% of mice [27]. In HNC patients, p53 mutations are associated with reduced survival and some evidence suggests that it may have a role in predicting clinical outcomes and response to treatment [28,29]. Recently, the Notch signaling pathway (NOTCH) and its ligand NOTCH1 have been found to be mutated in 10–15% of HNC, rendering it one of the most frequently mutated genes in HNC [30,31]. NOTCH1 appears to act as a tumor suppressor in HNC although some studies suggest that it may also exert an oncogenic effect [32]. To date, clinical trials using therapeutic interventions of Notch signaling are not available for HNC. However, as data from trials in other tumors emerge, future trials in HNC may be offered.

With the advent of large-scale tumor genome sequencing, novel oncogenes and tumor suppressor genes are emerging. Although these genes form the basis of essential therapies, patient responses to them remain incomplete, suggesting that the variability of host

responses and the tumor–host interaction play a fundamental role in determining tumor progression and patient responses [33].

IMMUNOSUPPRESSIVE CELLS IN THE MICROENVIRONMENT

The tumor microenvironment is infiltrated by a wide array of cells from both adaptive and innate immune systems. Based on their phenotypical and functional characteristics, these cells can be classified as antitumoral or protumoral. HNCs commonly demonstrate lymphocytic infiltration (Fig. 1). The presence of lymphocytes infiltrating the tumor has been described as a good prognostic indicator in some malignancies (Fig. 2) [34,35]. However, some lymphocyte subtypes can exert a protumoral effect. These cells promote tumor cell immune evasion by dampening the immune response and generating immune tolerance. They include regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAM). Tregs play a crucial role in tumor immune escape mechanisms and response to treatment in many malignancies [36,37]. They are a subset of CD4⁺ T cells that are characterized by cell surface expression of CD25 and forkhead box P3 which control key aspects of immunological tolerance to self-antigens [38,39]. Studies have demonstrated that there are increased Treg subsets in the peripheral blood of patients with laryngeal cancers compared with healthy donors [40]. Furthermore, there appears to be a positive correlation between the percentage of Treg cells and tumor size, suggesting that Tregs could be associated with disease prognosis [41]. MDSC are cells of the myeloid lineage that are comprised of myeloid-cell progenitors and precursors [42]. Under normal conditions, precursors of myeloid cells mature and differentiate into macrophages in tissue or dendritic cells in the circulation. In cancers, the population of precursors and progenitors are inhibited from differentiating and maturing; thus, there is an expansion of this population. The activation of these cells, in malignancy, results in an increased expression of immune suppressive factors, including nitric oxide and reactive oxygen species. Through direct inhibition of the cytotoxic functions of T cells and natural killer cells, MDSC are able to suppress both adaptive and innate immunity [43,44]. Recent studies have shown that in HNC, MDSC subsets express high levels of these immunosuppressive factors as well as the ability to induce Tregs [45]. TAMs are mature myeloid cells which comprise the predominant portion of leukocytes in the tumor microenvironment [46]. They possess an alternatively activated (M2) phenotype, which is characterized by production of tumor-promoting and immunosuppressive factors such as IL-6, IL-10, and EGFR [47]. TAMs in HNC have been associated with tumor progression, angiogenesis, and metastasis [48].

IMMUNOSUPPRESSIVE CYTOKINES IN THE MICROENVIRONMENT

One way in which tumor cells evade the immune response is by secreting a variety of cytokines and chemokines into the tumor microenvironment. These immunosuppressive cytokines negatively affect immune cell maturation and effector functions [49]. Vascular endothelial growth factor is secreted by tumor cells and acts to inhibit maturation and differentiation of dendritic cells through the suppression of NF- κ B in hematopoietic stem cells [50,51]. In cancers of the larynx, high vascular endothelial growth factor expression has been associated with poor disease-free survival [52]. Another important immunosuppressive

cytokine is IL-10. IL-10 is expressed by some immune cells and exerts an inhibitory effect on dendritic cell maturation and subsequent antigen presentation, which is crucial for successful T-cell activation [53–55]. IL-6 has been described as a multifunctional cytokine that plays a role in several cellular cycle processes, including the oncogenic *ras* pathway [56]. In cancers of the larynx, IL-6 appears to function as a suppressive cytokine, with studies demonstrating that serum levels are an independent predictor of progression-free survival [57]. Transforming growth factor- β (TGF- β) regulates many processes, including cell growth, differentiation, and apoptosis [58]. It is known to suppress natural killer and T-cell activation as well as aiding in the differentiation of Tregs [59]. Overexpression of TGF- β is commonly described in HNC and correlates with advanced disease and reduced overall survival [60]. In the larynx, TGF- β expression is found to be higher in cancers compared with dysplastic lesions and may be a useful marker to distinguish malignant transformation [61,62]. Within the tumor micro-environment immune cells encounter a variety of signals both from other immune cells and the tumor. The interaction between immunosuppressive cytokines and immune cells within the tumor microenvironment plays a significant role in the transformation of laryngeal dysplasia to malignancy.

IMMUNE CHECKPOINT RECEPTORS

For T cells to become activated they require engagement of both their receptor and costimulatory molecules. Immune checkpoints function to regulate the extent of this activation and to prevent excessive activation and autoimmunity. These inhibitory pathways also play a role in the tumor microenvironment and have been employed by tumor cells as a mechanism of immune evasion [63]. The ligation of immune checkpoint receptors by their respective ligands mediates these inhibitory pathways. Commonly studied receptor-ligand combinations include programmed death ligand-1 (PD-1) and its ligands PD-L1 and PD-L2 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) and its ligands CD80 and CD84. Recently, Vassilakopoulou *et al.* [64] demonstrated increased PD-L1 expression in primary laryngeal carcinomas compared with surrounding normal tissue. Interestingly, this correlated with increased density of tumor infiltrating lymphocytes and improved disease-free survival. A correlation between expression of CTLA-4 on T cells in the peripheral blood and aggressive tumor growth in patients with laryngeal cancer has also been described by several studies [65–69]. Future antitumor therapies may consider these neoantigens in the context of genetic diversity to develop a combined approach to improve therapy.

CONCLUSION

The classical tumor progression model describes the accumulation of multiple mutations in tumor suppressor genes and oncogenes which, in combination with environmental carcinogens, results in the accumulation of DNA damage and subsequent malignant transformation of cells. Although this model is supported by the discovery of multiple mutations in laryngeal cancers, they do not all contain these mutations, suggesting that an additional factor is involved in tumorigenesis. It has become increasingly evident that host-tumor reactivity, mediated by immune cells in the tumor microenvironment, contributes significantly to the variability in tumor development. This suggests that, aside from direct visualization and biopsy of suspicious lesions involving the larynx to identify malignant

cells, it may be valuable to evaluate the presence of immune/inflammatory infiltrating cells within dysplastic lesions. This so-called ‘immunoscore’ quantifies the immune cellular infiltrate as well as basic phenotypic information, to reflect the extent of host responses. Correlation of this information with progression to malignancy would aid the field, but has not yet been done in many pre-malignant lesions. Additionally, further studies to identify biomarkers of malignant transformation, including immunosuppressive soluble mediators (cytokines) may be beneficial to guide diagnosis and less invasive, targeted therapy. Targeting the tumor microenvironment, in combination with genetic targets, to reverse tumor immune escape and reprogram inhibitory immune cells are key areas for novel antitumor therapies.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■ ■ of outstanding interest

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin.* 2015; 65:5–29. [PubMed: 25559415]
2. Leoncini E, Vukovic V, Cadoni G, et al. Clinical features and prognostic factors in patients with head and neck cancer: results from a multicentric study. *Cancer Epidemiol.* 2015; 39:367–374. [PubMed: 25770642]
3. Gale N, Zidar N, Poljak M, Cardesa A. Current views and perspectives on classification of squamous intraepithelial lesions of the head and neck. *Head Neck Pathol.* 2014; 8:16–23. [PubMed: 24595419]
4. Bouquot JE, Gnepp DR. Laryngeal precancer: a review of the literature, commentary, and comparison with oral leukoplakia. *Head Neck.* 1991; 13:488–497. [PubMed: 1791144]
5. Weller MD, Nankivell PC, McConkey C, et al. The risk and interval to malignancy of patients with laryngeal dysplasia; a systematic review of case series and meta-analysis. *Clin Otolaryngol.* 2010; 35:364–372. [PubMed: 21108746]
6. Marcos CA, Alonso-Guervos M, Prado NR, et al. Genetic model of transformation and neoplastic progression in laryngeal epithelium. *Head Neck.* 2011; 33:216–224. [PubMed: 20629083]
7. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene.* 2008; 27:5904–5912. [PubMed: 18836471]
8. Bindea G, Mlecnik B, Angell HK, Galon J. The immune landscape of human tumors: implications for cancer immunotherapy. *Oncoimmunology.* 2014; 3:e27456. Comprehensive view of the role of the immune system in the course of tumor progression and recurrence. [PubMed: 24800163]
9. Brugere J, Guenel P, Leclerc A, Rodriguez J. Differential effects of tobacco and alcohol in cancer of the larynx, pharynx, and mouth. *Cancer.* 1986; 57:391–395. [PubMed: 3942973]

10. Cuchi A, Bombi JA, Avellaneda R, et al. Precancerous lesions of the larynx: clinical and pathologic correlations and prognostic aspects. *Head Neck*. 1994; 16:545–549. [PubMed: 7822176]
11. Hirabayashi H, Koshii K, Uno K, et al. Laryngeal epithelial changes on effects of smoking and drinking. *Auris Nasus Larynx*. 1990; 17:105–114. [PubMed: 2222330]
12. Altieri A, Garavello W, Bosetti C, et al. Alcohol consumption and risk of laryngeal cancer. *Oral Oncol*. 2005; 41:956–965. [PubMed: 15927525]
13. Talamini R, Bosetti C, La Vecchia C, et al. Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. *Cancer Causes Control*. 2002; 13:957–964. [PubMed: 12588092]
14. Lewin JS, Gillenwater AM, Garrett JD, et al. Characterization of laryngopharyngeal reflux in patients with premalignant or early carcinomas of the larynx. *Cancer*. 2003; 97:1010–1014. [PubMed: 12569600]
15. Li X, Gao L, Li H, et al. Human papillomavirus infection and laryngeal cancer risk: a systematic review and meta-analysis. *J Infect Dis*. 2013; 207:479–488. [PubMed: 23175764]
16. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*. 2013; 499:214–218. [PubMed: 23770567]
17. Gaykalova DA, Mambo E, Choudhary A, et al. Novel insight into mutational landscape of head and neck squamous cell carcinoma. *PloS One*. 2014; 9:e93102. Detailed overview of the mutational landscape of head and neck cancers, including novel mutated genes. [PubMed: 24667986]
18. Gale N, Kambic V, Poljak M, et al. Chromosomes 7,17 polysomies and overexpression of epidermal growth factor receptor and p53 protein in epithelial hyperplastic laryngeal lesions. *Oncology*. 2000; 58:117–125. [PubMed: 10705238]
19. Miyaguchi M, Olofsson J, Hellquist HB. Expression of epidermal growth factor receptor in laryngeal dysplasia and carcinoma. *Acta Otolaryngol*. 1990; 110:309–313. [PubMed: 2239223]
20. Ishitoya J, Toriyama M, Oguchi N, et al. Gene amplification and overexpression of EGF receptor in squamous cell carcinomas of the head and neck. *Br J Cancer*. 1989; 59:559–562. [PubMed: 2713242]
21. Shin DM, Ro JY, Hong WK, Hittelman WN. Dysregulation of epidermal growth factor receptor expression in premalignant lesions during head and neck tumorigenesis. *Cancer Res*. 1994; 54:3153–3159. [PubMed: 8205534]
22. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol*. 2010; 28:1075–1083. [PubMed: 20085938]
23. Bauman JE, Arias-Pulido H, Lee SJ, et al. A phase II study of temsirolimus and erlotinib in patients with recurrent and/or metastatic, platinum-refractory head and neck squamous cell carcinoma. *Oral Oncol*. 2013; 49:461–467. [PubMed: 23384718]
24. Sun W, Califano JA. Sequencing the head and neck cancer genome: implications for therapy. *Ann N Y Acad Sci*. 2014; 1333:33–42. [PubMed: 25440877]
25. Olivier M, Eeles R, Hollstein M, et al. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat*. 2002; 19:607–614. [PubMed: 12007217]
26. Gale N, Zidar N, Kambic V, et al. Epidermal growth factor receptor, c-erbB-2 and p53 overexpressions in epithelial hyperplastic lesions of the larynx. *Acta Otolaryngol Suppl*. 1997; 527:105–110. [PubMed: 9197495]
27. Ku TK, Nguyen DC, Karaman M, et al. Loss of p53 expression correlates with metastatic phenotype and transcriptional profile in a new mouse model of head and neck cancer. *Mol Cancer Res*. 2007; 5:351–362. [PubMed: 17426250]
28. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2007; 357:2552–2561. [PubMed: 18094376]
29. Temam S, Flahault A, Perie S, et al. p53 gene status as a predictor of tumor response to induction chemotherapy of patients with locoregionally advanced squamous cell carcinomas of the head and neck. *J Clin Oncol*. 2000; 18:385–394. [PubMed: 10637254]
30. Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science*. 2011; 333:1154–1157. [PubMed: 21798897]

31. Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science*. 2011; 333:1157–1160. [PubMed: 21798893]
32. Pickering CR, Zhang J, Yoo SY, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov*. 2013; 3:770–781. [PubMed: 23619168]
33. Shiga K, Ogawa T, Katagiri K, et al. Differences between oral cancer and cancers of the pharynx and larynx on a molecular level. *Oncol Lett*. 2012; 3:238–243. [PubMed: 22740888]
34. Al-Shibli KI, Donnem T, Al-Saad S, et al. Prognostic effect of epithelial and stromal lymphocyte infiltration in nonsmall cell lung cancer. *Clin Cancer Res*. 2008; 14:5220–5227. [PubMed: 18698040]
35. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012; 12:298–306. [PubMed: 22419253]
36. Cao X. Regulatory T cells and immune tolerance to tumors. *Immunol Res*. 2010; 46:79–93. [PubMed: 19763889]
37. Whiteside TL. Induced regulatory T cells in inhibitory microenvironments created by cancer. *Expert Opin Biol Ther*. 2014; 14:1411–1425. [PubMed: 24934899]
38. McHugh RS, Shevach EM. Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol*. 2002; 168:5979–5983. [PubMed: 12055202]
39. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev*. 2001; 182:18–32. [PubMed: 11722621]
40. Sun W, Li WJ, Fu QL, et al. Functionally distinct subsets of CD4(+) regulatory T cells in patients with laryngeal squamous cell carcinoma are indicative of immune deregulation and disease progression. *Oncol Rep*. 2015; 33:354–362. Novel study demonstrating a distinct subset of activated Tregs with suppressive activity in laryngeal squamous cell carcinoma. [PubMed: 25333227]
41. Dewyer NA, Wolf GT, Light E, et al. Circulating CD4-positive lymphocyte levels as predictor of response to induction chemotherapy in patients with advanced laryngeal cancer. *Head Neck*. 2014; 36:9–14. [PubMed: 23765859]
42. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009; 9:162–174. [PubMed: 19197294]
43. Almand B, Clark JI, Nikitina E, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol*. 2001; 166:678–689. [PubMed: 11123353]
44. Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, et al. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. *J Immunother*. 2012; 35:107–115. [PubMed: 22306898]
45. Chikamatsu K, Sakakura K, Toyoda M, et al. Immunosuppressive activity of CD14+ HLA-DR- cells in squamous cell carcinoma of the head and neck. *Cancer Sci*. 2012; 103:976–983. [PubMed: 22360618]
46. Mantovani A, Sozzani S, Locati M, et al. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol*. 2002; 23:549–555. [PubMed: 12401408]
47. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity*. 2014; 41:49–61. [PubMed: 25035953]
48. Lathers DM, Young MR. Increased aberrance of cytokine expression in plasma of patients with more advanced squamous cell carcinoma of the head and neck. *Cytokine*. 2004; 25:220–228. [PubMed: 15036248]
49. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of ‘tumor escape’ phenotypes. *Nat Immunol*. 2002; 3:999–1005. [PubMed: 12407407]
50. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood*. 1998; 92:4150–4166. [PubMed: 9834220]

51. Oyama T, Ran S, Ishida T, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. *J Immunol.* 1998; 160:1224–1232. [PubMed: 9570538]
52. Mineta H, Miura K, Ogino T, et al. Prognostic value of vascular endothelial growth factor (VEGF) in head and neck squamous cell carcinomas. *Br J Cancer.* 2000; 83:775–781. [PubMed: 10952783]
53. Sharma S, Stolina M, Lin Y, et al. T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *J Immunol.* 1999; 163:5020–5028. [PubMed: 10528207]
54. De Smedt T, Van Mechelen M, De Becker G, et al. Effect of interleukin-10 on dendritic cell maturation and function. *Eur J Immunol.* 1997; 27:1229–1235. [PubMed: 9174615]
55. Murray PJ. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr Opin Pharmacol.* 2006; 6:379–386. [PubMed: 16713356]
56. Horn F, Henze C, Heidrich K. Interleukin-6 signal transduction and lymphocyte function. *Immunobiology.* 2000; 202:151–167. [PubMed: 10993289]
57. Hao W, Zhu Y, Zhou H. Prognostic value of interleukin-6 and interleukin-8 in laryngeal squamous cell cancer. *Med Oncol.* 2013; 30:333. [PubMed: 23269580]
58. Massague J. TGFbeta in cancer. *Cell.* 2008; 134:215–230. [PubMed: 18662538]
59. Moutsopoulos NM, Wen J, Wahl SM. TGF-beta and tumors—an ill-fated alliance. *Curr Opin Immunol.* 2008; 20:234–240. [PubMed: 18486463]
60. White RA, Malkoski SP, Wang XJ. TGFbeta signaling in head and neck squamous cell carcinoma. *Oncogene.* 2010; 29:5437–5446. [PubMed: 20676130]
61. Kapral M, Strzalka B, Kowalczyk M, et al. Transforming growth factor beta isoforms (TGF-beta1, TGF-beta2, TGF-beta3) messenger RNA expression in laryngeal cancer. *Am J Otolaryngol.* 2008; 29:233–237. [PubMed: 18598833]
62. Franchi A, Gallo O, Sardi I, Santucci M. Downregulation of transforming growth factor beta type II receptor in laryngeal carcinogenesis. *J Clin Pathol.* 2001; 54:201–204. [PubMed: 11253131]
63. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell.* 2015; 27:450–461. [PubMed: 25858804]
64. Vassilakopoulou M, Avgeris M, Velcheti V, et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. *Clin Cancer Res.* 2015 Epub 2015/09/27. Study in laryngeal squamous cell cancer showing increased density of tumor infiltrating lymphocytes and PD-L1 levels are associated with better clinical outcomes.
65. Starska K, Forma E, Lewy-Trenda I, et al. Expression of CTLA-4 and Foxp3 in peripheral blood T cells of patients with squamous cell laryngeal carcinoma. *Contemp Oncol (Pozn).* 2013; 17:370–377. [PubMed: 24592125]
66. Erfani N, Khademi B, Haghshenas MR, et al. Intracellular CTLA4 and regulatory T cells in patients with laryngeal squamous cell carcinoma. *Immunol Invest.* 2013; 42:81–90. [PubMed: 23252863]
67. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med.* 2001; 345:1890–1900. [PubMed: 11756581]
68. Kerkar SP, Restifo NP. Cellular constituents of immune escape within the tumor microenvironment. *Cancer Res.* 2012; 72:3125–3130. [PubMed: 22721837]
69. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science.* 2006; 313:1960–1964. [PubMed: 17008531]

FIGURE 1.

Head and neck SCC, lymphocytic host response, and tumor infiltrative lymphocytes. (a) Left upper corner shows normal thickness of the oral tongue mucosa with keratosis. The mid and right thirds of the image show superficially invasive SCC with pushing invasive tumor front and band-like dense inflammatory infiltrate (i.e., lymphocytic host response), H&E, 100 $\times$. (b) Superficially invasive keratinizing SCC of the oral tongue demonstrating minimal lymphocytic host response (left upper corner), H&E, 40 $\times$. (c) Keratinizing invasive SCC of the oral tongue with more intense lymphocytic host response, H&E, 40 $\times$. (d) Tumor infiltrating lymphocytes in nonkeratinizing oropharyngeal SCC, H&E, 100 $\times$. H&E, hematoxylin and eosin; SCC, squamous cell carcinomas.

FIGURE 2.

Increased numbers of tumor infiltrating lymphocytes correlate with disease-free survival in CRC. Kaplan–Meier curves illustrating disease-free survival according to TNM stages [stage I, black line ($n = 75$ patients); stage II, gray line ($n = 137$); stage III, light gray line ($n = 99$), and stage IV, black line ($n = 95$)] in patients with CRCs. (a) A higher TNM stage correlates strongly with decreased disease-free survival. (b) After adjusting for TNM stage, those tumors with high infiltration of lymphocytes (CD3_{CT}^{Hi}CD3_{IM}^{Hi} plus CD45RO_{CT}^{Hi}CD45RO_{IM}^{Hi}, thin lines, $n = 88$) appear to correlate with increased disease-free survival. Log-rank statistical test, ** $P < 0.01$. CRC, colorectal carcinoma; ns, not significant; TNM, tumor nodes metastasis. Reproduced with permission from [69].