Seminars in Cancer Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Head and neck squamous cell carcinoma: Genomics and emerging biomarkers for immunomodulatory cancer treatments

Benjamin Solomon[a,b,c,*], Richard J. Young[b], Danny Rischin[a,c]

[a] Department of Medical Oncology, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria 3000, Australia
[b] Research Division, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria 3000, Australia
[c] Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria 3052, Australia

ARTICLE INFO

Keywords:
Human papilloma virus (HPV)
Head and neck squamous cell carcinoma
Genomics
p53
Programmed death 1 receptor (PD1 receptor)
Programmed death 1 receptor ligand 1 (PD-L1)

ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) comprises a heterogeneous group of tumors that arise from the squamous epithelium of the oral cavity, oropharynx, larynx and hypopharynx. While many HNSCCs are related to classical etiologic factors of smoking and alcohol, a clinically, genomically, and immunologically distinct subgroup of tumors arise from the epithelium of the tonsil and the base of tongue as a result of infection with Human Papilloma Virus (HPV). In this review we describe the genomic and immunologic landscape of HNSCC, highlighting differences between HPV-positive and HPV-negative HNSCC. While HPV-negative tumors are characterized by tobacco-associated mutations in genes including *TP53* and *CDKN2A*, in HPV-positive HNSCC integration of viral genome from HPV into the host cellular genome results in expression of the E6 and E7 viral oncoproteins, with consequent degradation of p53 and functional inactivation of Rb. The immune microenvironment of HNSCC is characterized by changes in immune cell populations, immune checkpoints, as well as tumor or microenvironmental factors that alter the balance of the immune milieu in favor of immunosuppression, allowing tumor evasion and escape from immune surveillance. Immune therapies, in particular those targeting the PD1 receptor or its ligand PD-L1, including nivolumab, pembrolizumab, durvalumab, and atezolizumab have shown significant efficacy in subsets of patients with HNSCC. Current trials are evaluating the efficacy of these agents in combination with chemotherapy, radiotherapy and other immune therapies including CTLA-4 and IDO-1 inhibitors. While biomarkers including PD-L1 expression, PD-L2 expression and the interferon-gamma gene signature show potential to predict benefit from checkpoint inhibitor therapy – it is hoped that improved understanding of the genomic and immune landscape will lead to ways to improved strategies to stratify patients and to select which HNSCC are most likely to benefit from these therapies.

1. Introduction

Head and neck cancers are the 6th most common malignancy worldwide accounting for about 600,000 new cases per year [1,2]. Head and neck squamous cell carcinoma (HNSCC) comprises the majority of head and neck cancers, and represent a heterogeneous group of tumors that arise from the squamous epithelium of the oral cavity, oropharynx, larynx and hypopharynx. Many HNSCC patients present with locally advanced disease, often with prominent involvement of lymph nodes. Conventional treatments for locally advanced disease are multimodal, utilizing combinations of surgery, radiotherapy and chemotherapy, result in significant short term and long term morbidity, and are curative in only about 50% of patients.

HNSCC can be divided into two distinct subgroups based on Human Papilloma Virus (HPV) status. While HPV-negative HNSCCs are related to the environmental carcinogens tobacco and alcohol, HPV-positive HNSCC is a result of HPV infection, predominantly by the HPV-16 subtype. HPV-positive HNSCC arises in the oropharynx from within the lymphoid associated epithelium of the tonsil or base of tongue, typically occur in younger patients who are often non-smokers. HPV-positive HNSCC is associated with improved prognosis compared with HPV-negative HNSCC [3–5]. There has been a marked increase in the incidence of HPV-positive HNSCC which now represents the most common form of oropharyngeal cancer in many western populations [6,7].

Apart from HPV status, to date there are a paucity of robust, clinically useful predictive or prognostic biomarkers in HNSCC. Recent comprehensive genomic analyses have provided insights into the genomic landscape of HNSCC showing important differences, but also overlapping features between HPV-positive and HPV-negative HNSCC.

* Corresponding author at: Department of Medical Oncology, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria 3000, Australia.
E-mail address: ben.solomon@petermac.org (B. Solomon).

https://doi.org/10.1016/j.semcancer.2018.01.008
Received 19 October 2017; Received in revised form 29 December 2017; Accepted 13 January 2018

And although the immunosuppressed nature of the tumor microenvironment has long been appreciated, there has been renewed enthusiasm for characterizing the immune landscape of HNSCC given significant clinical activity of immunotherapy approaches in particular PD1 and PD-L1 inhibitors. In this review we describe insights into the genomic and immune landscape of HNSCC together with clinical advances in immune therapy for HNSCC, in the context of HPV status, and explore prospects for use of biomarkers to prognostically stratify and select patients for therapy. Nasopharyngeal carcinoma, which represent a distinct subgroup of head and neck cancers, often associated with Epstein-Barr Virus infection, are not included in this overview.

2. Genomic landscape of HNSCC

Genomic studies have revealed a complex landscape in HNSCC, encompassing frequent chromosomal changes, DNA copy number alterations, somatic mutations and promoter methylation [8–14] with significant spatial and temporal heterogeneity [15–17]. Whole genome sequencing studies have demonstrated that HPV-negative HNSCC, which is related to classical etiologic factors of tobacco and alcohol, closely resemble lung squamous cell carcinoma [8]. In contrast, HPV-positive HNSCC is clinically and genomically distinct (Table 1). Integration of viral genome from HPV into the host cellular genome, and expression of the E6 and E7 viral oncoproteins, results in degradation of p53 together with functional inactivation of Rb. These HPV-positive tumors occur in younger patients, often in the absence of a history of tobacco consumption. Distinctive and overlapping genomic features have been identified between HPV-positive and HPV-negative HNSCC [8–11].

Complex chromosomal changes and gene copy number alterations are present across the spectrum of HNSCC and are evident even in premalignant disease. HPV-negative tumors have more frequent chromosomal changes, including losses of 3p and 8p and gains of 3q, 5p and 8q [9–12,18,19]. HPV-negative tumors have coamplification of 11q13 (containing *CCND1*, *FADD*, and *CTTN*) and 11q22 (containing *BIRC2* and *YAP1*). Focal gains of *EGFR, REL, BCL6, PIK3CA, TP63, CCDN1*, and *MDM2* [11,20] and losses of *ATM, CDKN2A, RB1, NOTCH1* and *NF1* [11] are seen more often in HPV-negative tumors. Other changes, such as amplification of chromosome 3q26-28 region containing *TP63*, *SOX2*, and *PIK3CA,* are seen in both HPV-positive and HPV-negative tumors. HPV-positive tumors have recurrent deletions of TNF receptor associated factor 3 (*TRAF3*) (a gene implicated in NF-κB signaling and immune responses to virus) [18], focal amplification of *E2F1* [18] and in contrast to HPV-negative HNSCC lack deletions in 9q21.3 region containing *CDNK2A* gene [18].

Recent large-scale whole genome sequencing studies, including the cancer genome consortium (TCGA), have confirmed frequent somatic mutations of genes, including *TP53, CDKN2A, PTEN, PIK3CA,* and *HRAS,* and also revealed novel recurrent mutations such as those involving the NOTCH signaling pathway [9,10,18]. In contrast to tumor types such as lung adenocarcinoma and melanoma, there are however a relative paucity of therapeutically actionable genes. While some early studies have suggested HPV-negative tumors have more mutations than HPV-positive tumors [9,10], other more recent studies have indicated that mutation rate does not significantly differ by HPV status [11,18]. Mutational signatures associated with transversions at CpG sites, associated with tobacco [21] are more frequent in HPV-negative tumors, while a predominance of TpC mutations associated with the APOBEC signature [21,22] are seen in HPV-positive tumors [11,17,18]. Mutations are more frequent identified in tumors from smokers compared with non-smokers in both HPV-positive and HPV-negative HNSCC [11,17,18].

The most frequently identified somatic mutations in head and neck cancer are mutations involving the tumor suppressor genes *TP53* and *CDNK2A*. Mutations involving *TP53,* the gene that encodes p53, are highly prevalent in HPV-negative tumors. While these are predominantly inactivating or loss of function mutations, gain of function mutations can also occur. *TP53* mutations, in particular disruptive mutations, have been associated with poor prognosis and resistance to therapy [23]. In contrast, *TP53* mutations are rare in HPV-positive tumors, where the E6 viral oncoprotein mediates ubiquitinylation and proteasomal degradation of p53. *CDKN2A* the gene that encodes p16 regulates cell cycle progression by blocking the activity of CCND1 (cyclin D1) and the cyclin-dependent kinases, CDK6 and CDK4, which phosphorylate and inactivate the tumor suppressor RB1. *CDNK2A* may be disrupted by somatic mutations, deletion or promoter hypermethylation [24], often in the context of amplifications of *CCND1* (the gene that encodes Cyclin D1) [12]. Data from the TCGA indicate that 28% of HNSCC had *CCND1* amplification (32% (77/243) HPV-negative and 6%(2/36) HPV-positive tumors). Overexpression of Cyclin D1 and amplification of *CCND1* have been associated with poor prognosis and resistance to therapies including EGFR inhibitors.

Whole-exome sequencing studies have identified that over one-third of tumors have mutations in genes involved in the regulation of

Table 1
Clinical and Molecular features of HPV-positive and HPV negative Head and Neck Squamous Cell Carcinoma (HNSCC). Adapted from Hammerman et al. [113].

	HPV-negative HNSCC	HPV-positive HNSCC
Clinical Features		
Site	Oral cavity, oropharynx, larynx, hypopharynx	Predominantly oropharynx (Tonsil and Base of Tongue)
Age	Older (typically 7th decade of life)	Younger(typically 6th decade of life)
Gender	Male 3:1	Male 3:1
Risk Factors	Smoking, Alcohol, Betel nut consumption	Sexual Behaviours
Histology	Keratinized	Basaloid
Molecular Features		
TP53	Mutation or loss almost universal	HPV mediated functional loss via E6
Cell cycle deregulation (CDNK2A and CCND1)	Loss of *CDNK2A/RB1* by mutation, deletion or methylation. *CCNS1/CDK4/CDK6* amplification common	Loss of Rb via HPV E7; E2F1 amplification
Copy number gain	3q26-28, *EGFR, FGFR1*, 11q13	3q26-28
Copy number loss	*CDKN2A*, 3p, *UBR5*	*ATM*
PI3K pathway activation (including PIK3CA amplification/mutation)	In ~30%	In ~50%
RTK mutations/amplifications/fusions	*EGFR* and *FGFR1* amplification	*FGFR2/3* mutations in > 10%; Rare *FGFR3-TACC* Fusion
RAS	5–10% (*HRAS* more common)	5–10% (*KRAS* more common)
Immune evasion genes	Rare HLA mutations	*HLA, B2M*, and *TRAF* loss
Oxidative Stress Regulation	Common activation of *NFE2L2/KEAP1/CUL3* (25%)	rare
Differentiation	Loss of *NOTCH1/FAT1/AJUB*; *TP63* gain	Loss of *NOTCH1; TP63* gain