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Research Paper
Head and Neck Oncology

Tumor PD-L1 expression is associated with improved survival and lower recurrence risk in young women with oral cavity squamous cell carcinoma

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Abstract. Young patients with oral cavity squamous cell carcinoma (OCSCC) are often recognized as a distinct epidemiological cohort. In this study, genomic and immune-based metrics were correlated with long-term outcomes for a young patient population treated at a single institution. A fully clinically annotated, retrospective cohort of 81 patients aged ≤45 years with OCSCC is described, and the impact of clinicopathological features on long-term survival outcomes is reported. Genomic and immune parameters were integrated utilizing a whole-exome sequencing and immunohistochemical approach among females in the cohort. It was found that young OCSCC patients had favorable outcomes (10-year disease-free survival 79.1%, overall survival 80.0%) regardless of sex, particularly if they presented with oral tongue primaries and early stage disease. While mutational analysis appeared similar to that of older patients with OCSCC who lack a smoking history, a comparatively high degree of PD-L1 expression and PD-1/L1 concordance ($P = 0.001$) was found among young female OCSCC patients. Subjects with greater membranous PD-L1 positivity and the presence of tumor-infiltrating lymphocytes had a decreased risk of recurrence ($P = 0.01$ and $P = 0.01$, respectively) and improved survival ($P = 0.04$ and $P = 0.03$, respectively). These findings warrant further validation and support the investigation of immunotherapeutic approaches targeting PD-1/L1 interactions in young OCSCC patients.

Key words: PD-L1; biomarkers; young patients; head and neck cancer; survival.

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While head and neck cancers primarily occur in older adults, a unique subset of patients develop oral cavity squamous cell carcinoma (OCSCC) at a young age, particularly of the oral tongue[1]. The incidence of OCSCC in patients between the ages of 18 and 44 years appears to be increasing, particularly in young women[2–4]. Despite a clear association between oncogenic

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strains of the human papillomavirus (HPV) and the development of oropharyngeal cancers of the head and neck, OCSCC is infrequently associated with HPV infection[5,6]. These young patients typically lack exposure to traditional risk factors such as tobacco and alcohol, but even when young OCSCC patients report exposure, the estimated attributable risk appears low[7]. This has led investigators to hypothesize alternative causation, such as genetic abnormalities, viral or microorganism carcinogenesis, or other environmental exposures. However, to-date the only evidence supporting the distinctness of this group remains epidemiological.

Earlier studies suggested that the prognosis may be worse in young patients with OCSCC, but more recent retrospective, long-term data have indicated that their outcomes collectively are similar to those of older patients with this disease[3,8,9]. Despite these conflicting data, it remains important to consider that differences may exist in this unique subgroup of patients when making treatment recommendations. Conventional surgery, radiation, and chemotherapy can impose lasting functional limitations and chronic adverse sequelae in this young population.

Genomic characterization efforts involving a young OCSCC patient population have shown that gene-specific mutation and copy-number alteration frequencies are similar to those in older patients with this disease, and often lack a mutational signature associated with tobacco exposure[10,11]. While genomic data are of critical importance in understanding the molecular pathogenesis of squamous cell carcinoma of the head and neck (SCCHN), recent clinical success with immune checkpoint receptor blockade, namely anti-programmed cell death 1 (PD-1) therapy, provides evidence that understanding the characteristics of the tumor immune microenvironment is critical to informing treatment strategies. It is now widely recognized that tumor cells harbor immune checkpoint ligands to evade immune recognition[12]. The ligand of PD-1 (PD-L1) is variably expressed by head and neck tumor cells, and immunotherapies that block inhibitory immune cell signaling have demonstrated clinical efficacy in advanced head and neck cancers[13,14]. Work to understand the relationship between the mutational landscape and tumor–immune interactions is underway. In this study, genomic data and immune-based metrics were integrated with long-term outcomes in young women with OCSCC with the aim of identifying prognostic features and opportunities for therapeutic intervention.

Materials and methods

Study population

Young male and female patients aged ≤45 years at initial diagnosis, with biopsy-proven OCSCC, who had received treatment at the Dana-Farber Cancer Institute in Boston, USA between 1993 and 2012, were identified retrospectively following institutional review board approval. The age designation of 45 years or less is consistent with previous reports in the literature[7]. Patient demographic characteristics, clinicopathological features, and treatment outcomes were recorded. HPV status was reported if evaluated, although testing is no longer standard at the study institution for OCSCC tumors.

Immune-based metrics

Excisional tumor biopsies from patients consisted of the primary tumor or excised regional lymph nodes. Immunohistochemical studies with previously validated, in-house analytic PD-L1 (clone 9A11, mouse antibody) and PD-1 (clone EH33, mouse antibody) antibodies were used to characterize tissue slides prepared from formalin-fixed, paraffin-embedded (FFPE) tissue blocks[15]. Independent pathological review of hematoxylin–eosin-stained slides was performed to confirm the presence of tumor in each sample. An expert oral and maxillofacial pathologist blinded to the clinical and survival data analyzed the presence and distribution of membranous and cytoplasmic tumoral PD-L1 immunoreactivity. The intensity of PD-L1 expression was quantified using the *H*-score, which comprises a combination of both the intensity (scored as 0, 1+ (weak), 2+ (moderate), or 3 + (strong)) and the percentage of positive-stained tumor cells, as described previously[16]. The presence of tumor-infiltrating lymphocytes (TILs) was also quantified: scored as 0, 1+ (weak), 2+ (moderate), or 3+ (strong). Immunoreactivity for PD-1 was evaluated among TILs, with the absolute number of PD-1-positive TILs counted under 40× middle-power field, as described previously[16]. PD-L1 expression was also evaluated among TILs. For each slide, five representative areas were counted and the average absolute number was recorded.

Whole-exome sequencing (WES) and genomic analysis

WES was performed on DNA extracted from FFPE archival tumor samples. Briefly, DNA was sequenced following Illumina ICE hybrid-capture to a minimum coverage of 100×. Variant calling (single nucleotide variants (SNVs), indels) was performed using the Firehose pipeline running MuTect and filtering out OxoG artifacts. Using the Oncotator annotation tool (http://www.broadinstitute.org/oncotator/), likely germline mutations that were seen previously in both dbSNP Build 134 and 1000 Genomes data, or that had a population minor allele frequency ≥1% in NHLBI Exome Sequencing Project GO exomes, were removed.

The significance analysis was conducted using three versions of MutSig (MutSig 2CV, MutSig 2.0, and MutSig 1.5) that have different methods for calculating background mutation rates. To estimate this background, MutSig 1.5 uses the synonymous mutation rate, MutSig 2.0 uses the enrichment of mutations at evolutionarily conserved positions and the clustering of hotspot mutations, and MutSig 2CV incorporates gene expression, replication time, and chromatin state. Taking a stringent approach, it was required that genes reached statistical significance (q-value of ≤0.25) using all three methods. Since the dataset contained a mixture of patient-matched tumor–normal and unmatched tumor–normal pairs, only genes with at least one recurrent mutation in a matched tumor–normal pair were considered. Finally, genes that are not expressed in head and neck tumors were removed by downloading The Cancer Genome Atlas (TCGA) RNA-seq expression data for 546 SCCHN tumor samples from the NCI Genome Data Commons[17] and removing genes that showed <0.5 FPKM expression values across >90% of the samples.

Copy number analysis was performed using ReCapSeg (http://gatkforums.broadinstitute.org/gatk/discussion/5640/recapseg-overview), which compares each tumor sample to a panel of normal samples with similar library preparation methods. ReCapSeg determined segmented regions of $\log_2$-transformed copy number ratios, and annotated gene segments using Oncotator[18]. Recurrent copy number changes were detected using a GISTIC 2.0[19] search for peaks of copy number recurrence across the exome.

Pathogen analysis was performed using the PathSeq algorithm[20], which first removed human reads, then aligned residual reads to databases of human and microbial genomes. The microbial reads were then sorted into their taxonomic classifications.

Statistical analysis

Differences between individual subgroups were assessed using Fisher's exact test for categorical variables and the Wilcoxon

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