

Background

There has been growing interest in the role of the immune microenvironment in the development and behavior of head and neck squamous cell carcinoma (HNSCC)¹⁻⁴. It has been hypothesized that the efficacy of standard treatments (e.g., radiation and cisplatin), and the potential for immunotherapy, may depend partly on an immune response in addition to standard cytotoxic activity¹⁻⁵. Recently, several bioinformatic deconvolution methods have been developed to estimate the predominance of specific immune cell populations from transcriptome data derived from whole tumor samples⁵.

Objectives

Here, we sought to first validate previously reported trends in immune cell environment in HPV-driven oropharyngeal SCC to non-HPV driven HNSCC. Next, we sought to identify trends in immune cell environment in individuals who remained free of local recurrence to those with disease recurrence.

Methods and Materials

Single sample gene set enrichment analysis was utilized to carry out deconvolution of the immune population in gene expression data derived from an existing HNSCC gene expression data set (Illumina HumanHT-12-v3 Expression BeadChip Arrays) at Montefiore Medical Center (MMC). Results were additionally characterized using alternative bioinformatics tools (cibersort and ESTIMATE). Findings were then validated using the same methods carried out on transcriptome data from The Cancer Genome Atlas (TCGA). Median scores representing the relative abundance of immune cells were compared using Wilcoxon rank-sum tests. The immune profile was compared between HPV-associated oropharynx cancers and other HNSCCs identified by PCR detection of HPV. The relationship between immune profile and locoregional recurrence was examined using logistic regression models built using the MMC cohort.

Results

Table I. Characteristics of the Montefiore Medical Center cohort (MMC; N=179) and The Cancer Genome Atlas cohort (TCGA; N=280).

Characteristics*	Montefiore N = 179	TCGA N = 280	Total N = 459	P-Value ^b
Age (yr)	62.8 (11.8) (56-70)	60.9 (11.9) (53-68)	60.9 (12.0) (53-69)	0.011
Gender				0.003
Female	51 (29.3%)	79 (28.6%)	173 (29.4%)	
Male	123 (70.7%)	197 (71.4%)	415 (70.6%)	
Race				
White	116 (68.2%)	242 (87.7%)	462 (79.2%)	
Black	52 (30.0%)	28 (10.1%)	112 (19.3%)	
American Indian/Alaskan Native	0 (0.0%)	1 (0.4%)	2 (0.3%)	
Asian	2 (1.2%)	5 (1.8%)	7 (1.2%)	
Smoking History, ever vs never				
Never Smoker	26 (14.9%)	55 (20.3%)	108 (18.6%)	
Smoking	148 (85.1%)	216 (79.7%)	473 (81.4%)	
Smoking History, over 10 pack-yr	165 (94.8%)	272 (97.1%)	546 (92.2%)	<0.001
Smoking History, (Pack Years)	48.2 (53.4)	51.8 (57.3)	45.8 (55.5)	<0.001
Alcohol History, any vs never	41 (23.6%)	45 (16.4%)	40 (22.6%)	<0.001
No History	69 (41.6%)	93 (33.2%)	248 (42.6%)	
Alcohol Use	107 (60.4%)	187 (66.8%)	334 (57.4%)	
Human Papilloma Virus (HPV) Status				
Negative	98 (58.7%)	234 (86.9%)	456 (77.9%)	
Positive	69 (41.3%)	46 (16.4%)	129 (22.1%)	
Site of Cancer				
Larynx	48 (27.6%)	146 (52.1%)	249 (42.1%)	
Larynx/Hypopharynx	61 (35.1%)	76 (27.1%)	150 (26.3%)	
HPV + Oropharynx	38 (21.8%)	27 (9.8%)	78 (13.3%)	
HPV - Oropharynx	25 (14.4%)	31 (11.1%)	107 (18.1%)	
HPV Unknown	2 (1.1%)	0 (0.0%)	2 (0.3%)	
Oropharynx	0 (0.0%)	0 (0.0%)	5 (1.0%)	
Tumor Staging				
T1	30 (17.6%)	14 (5.0%)	57 (10.1%)	
T2	48 (28.2%)	84 (30.1%)	159 (28.1%)	
T3	38 (22.4%)	84 (30.1%)	147 (26.0%)	
T4	54 (31.8%)	97 (34.8%)	203 (35.9%)	
Node Staging				
N0	73 (42.4%)	133 (48.0%)	257 (45.4%)	
N1	30 (17.6%)	49 (16.6%)	81 (14.3%)	
N2	72 (41.9%)	90 (32.5%)	208 (36.7%)	
N3	7 (4.1%)	8 (2.9%)	20 (3.5%)	
Metastasis Staging				
M0	170 (98.8%)	277 (98.9%)	447 (98.9%)	
M1	1 (0.6%)	3 (1.1%)	4 (0.9%)	
MX	1 (0.6%)	0 (0.0%)	1 (0.2%)	
Disease-Free Survival Time (months)	35.7 (13-99.4)	34.1 (13-99.4)	37.9 (13-99.4)	<0.001

*Continuous variables were described using mean (± standard deviation) and median (interquartile range). Frequencies of categorical variables were reported as number (proportion of overall group within column).
*P-Values for both an analysis of variance (ANOVA) and Kruskal-Wallis test were reported for continuous variables. Chi-Square tests were used to assess categorical variables.
*Human Papilloma Virus status was assessed using p16 protein expression for MMC subjects, in situ hybridization using the INFORM HPV III Family 16 Probe for UICC subjects, and >1,000 mapped RNA sequencing (RNA-Seq) reads for TCGA subjects.

The MMC cohort consisted of 179 subjects predominantly white (68.2%) males (70.5%) with a median age of 62.9 at diagnosis (IQR 56-70). The group contained tumor of diverse primary sites – oral cavity (26.8%), larynx and hypopharynx (33.9%), and oropharynx (39.4%). Of the oropharynx cases, 55.6% were HPV positive. Median disease-free survival for the cohort was 35.5 months (IQR 13.7-98.5).

Immune infiltration differed between subjects with HPV-driven HNSCC of the oropharynx compared to all other sites. In both MMC and TCGA patients, overall immune cell presence, overall T-cell infiltration, and CD8+ T cells were higher among HPV-driven disease (p<0.05 all). CTLA4 expression was also higher among HPV-driven disease.

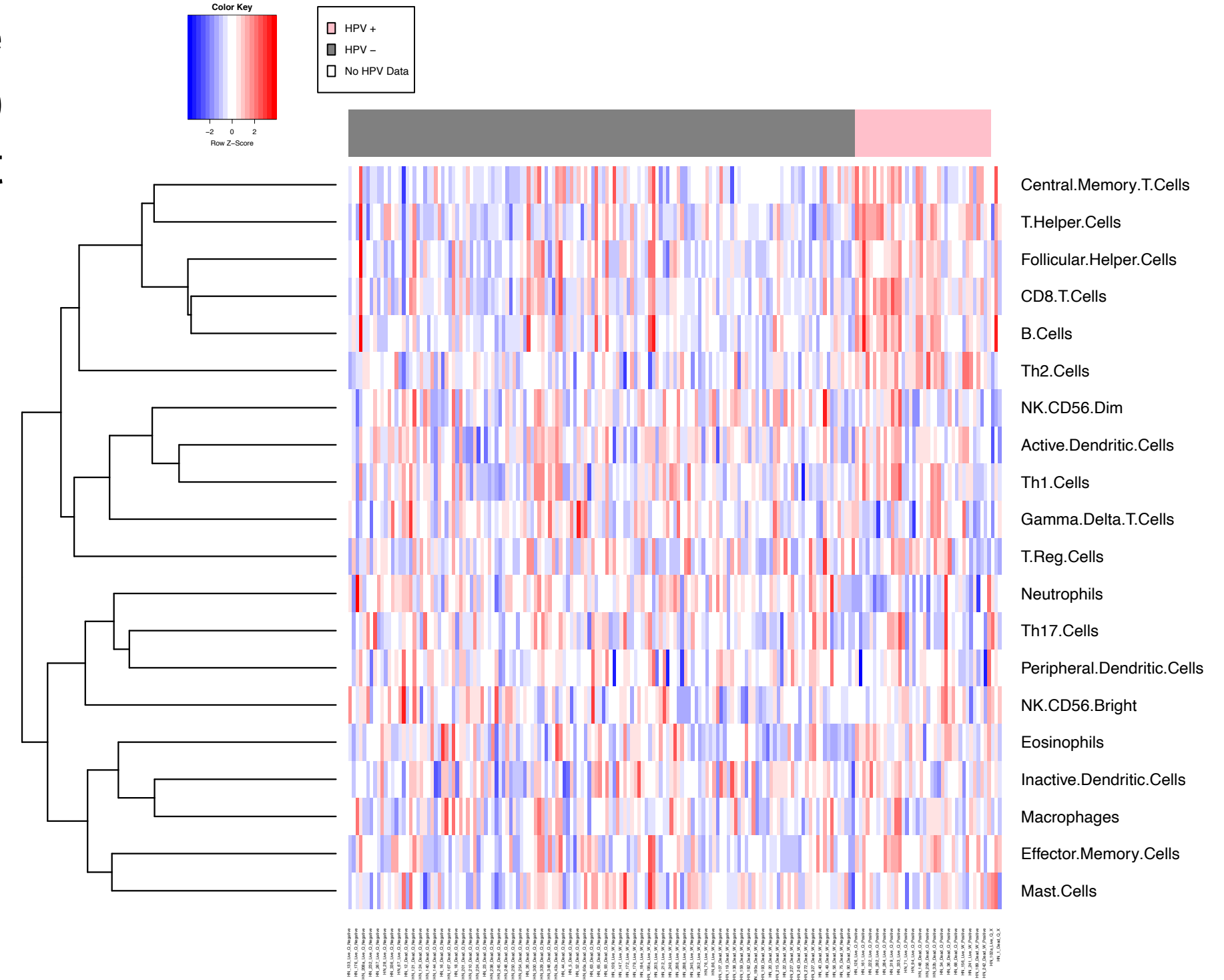


Figure 1. Heat map of relative abundance of different immune cell types by HPV status.

Results, continued

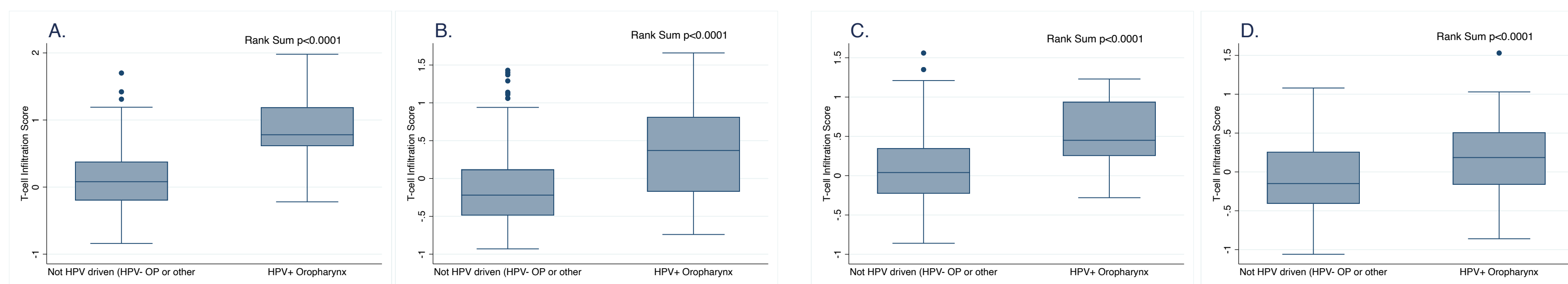


Figure 1. Box graphs of relative abundance of immune cell populations in patients with HPV-driven oropharyngeal squamous cell carcinoma as compared to those with non-HPV driven oropharyngeal cancer or head and neck squamous cell carcinoma as of other sites. **A.** demonstrates difference in median presence of all immune cell types by HPV status in the MMC cohort **B.** difference in median presence of all immune cell types by HPV status in the TCGA. **C.** difference in median presence of all T cells by HPV status in MMC cohort. **D.** difference in median presence of all T cell types by HPV status in the TCGA.

Among individuals that developed a disease recurrence, median T cell score was lower, though this difference did not reach significance (p=0.051). Median NK CD56 dim cells levels were lower among those with documented recurrence (p=0.048). CTLA4 levels were also higher among those with no recurrence (p=0.02). Univariate logistic regression in the MMC cohort found significant associations between several immune cell populations and recurrence of disease. Relative abundance of CD8+ T cells (OR -9.3, p<0.001), and effector memory T cells (OR -3.8, p=0.007) were associated with decreased odds of recurrence. Relative deficiency of Type 1 CD4+ T cells, macrophages, dendritic cells, NK CD56 bright cells and NK CD56 dim cells were associated with increased odds of recurrence (p<0.05 for all). Lower CTLA and PDL1 Levels were also associated with increased odds of recurrence (p<0.05).

Conclusion

- ❖ There were several consistent characteristics of the immune cell population related to both HPV-driven disease and lack of disease recurrence, suggesting that these key features in the immune microenvironment may contribute to treatment response and survival.

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