Differential Immune Responses to HPV-positive and HPV-negative Head and Neck Cancer

Hannan Qureshi, MD[1,2]; Xiaodong Zhu[2], Grace H. Yang[2], Melissa Shipley[2], Robert H. Pierce[2], Neal D. Futran[1], Sylvia M. Lee[2], Eduardo Méndez[1,2], A. McGarry Houghton[2]
[1]University of Washington, [2]Fred Hutchinson Cancer Research Center

BACKGROUND

Immune checkpoint inhibitors (ICIs) have been effective in achieving durable responses in head and neck squamous cell carcinoma (HNSCC); however, less than 20% of patients benefit from even the most promising ICI drugs. There is a lack of accurate predictors for response to ICI treatment in HNSCC and little is known about which types of immune cells have a more dominant impact on T-cell infiltration in HPV-positive and HPV-negative HNSCC. The main objective of our study is to identify the unique immune signatures of HPV-positive and HPV-negative HNSCC and to determine the major immune suppressive entities within their respective tumor microenvironments (TME).

METHODS

- Retrospective analysis of two separate cohorts of HNSCC patients:
  - Resection cohort: 102 HNSCC patients (44 HPV+, 58 HPV-)
  - ICI treatment cohort: 24 HNSCC patients (4 HPV+, 20 HPV-).
- Immune signature by HPV-status was determined using multiplex gene expression analysis (NanoString PanCancer Immune Profiling Panel).
- Multiplex immunohistochemistry (MIHC) was used to simultaneously evaluate markers for macrophages (CD68/CD163), neutrophils (CD66b), cytotoxic T-cells (CD8), B-cells (CD19/CD20), tumor cells (cytokeratin), PD-L1 expression, and DAPI (nuclear stain).
- Immune cell distributions and their spatial relationships were quantified with HALO image analysis software (Indica Labs).
- Second MIHC panel developed to focus specifically on B and T cell populations in ICI treatment cohort using markers of CD4 and CD8 T cells, CD19 and CD20 B cells, and cytokeratin for tumor cells.

RESULTS

- NanoString analysis revealed increased differential expression of genes related to B-cell functions in HPV-positive HNSCC, such as MS4A1, CD79B, and CD27, which were significant at a BY adjusted *p*-value of <0.001. T-cell function genes (e.g. CTLA4 and LAG3) also had significantly increased expression in HPV-positive HNSCC compared to HPV-negative HNSCC.
- MIHC in the resection cohort displayed increased CD8$^+$ T-cell, CD19/CD20$^+$ B-cell, and CD68/CD163$^+$ macrophage densities in the tumor region of HPV-positive HNSCC as opposed to HPV-negative HNSCC ($p < 0.001$).
- MIHC for the ICI treatment cohort again showed trends towards higher B cells in the HPV-positive TME, but this did not reach statistical significance ($p > 0.05$). Furthermore, the increased B cell content also trended towards improved PFS for HPV-negative HNSCC receiving ICI therapy ($p > 0.05$).

RESULTS

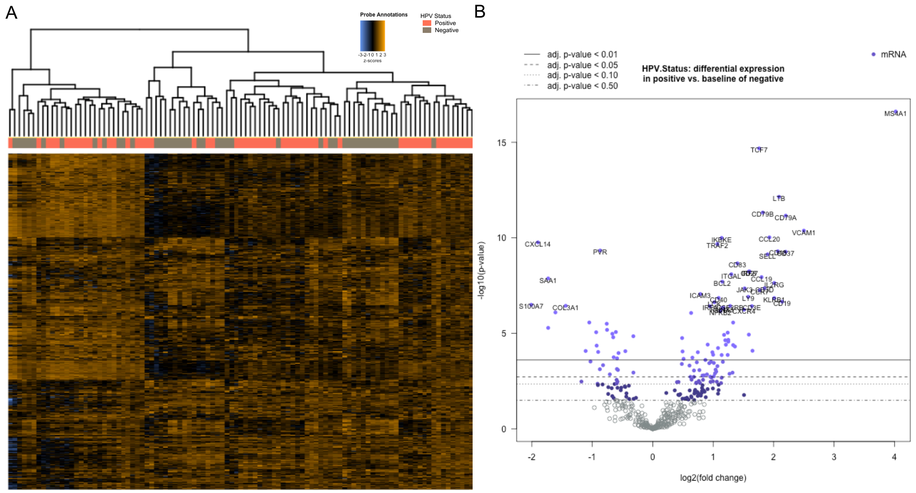

Figure 1: Differential expression of immune function-related genes in HPV-positive and HPV-negative HNSCC. (A) Heatmap generated via unsupervised clustering displaying differential gene expression patterns in HPV-positive and HPV-negative HNSCC. (B) Volcano plot showing significantly increased expression of B-cell and T-cell function related genes in HPV-positive HNSCC.

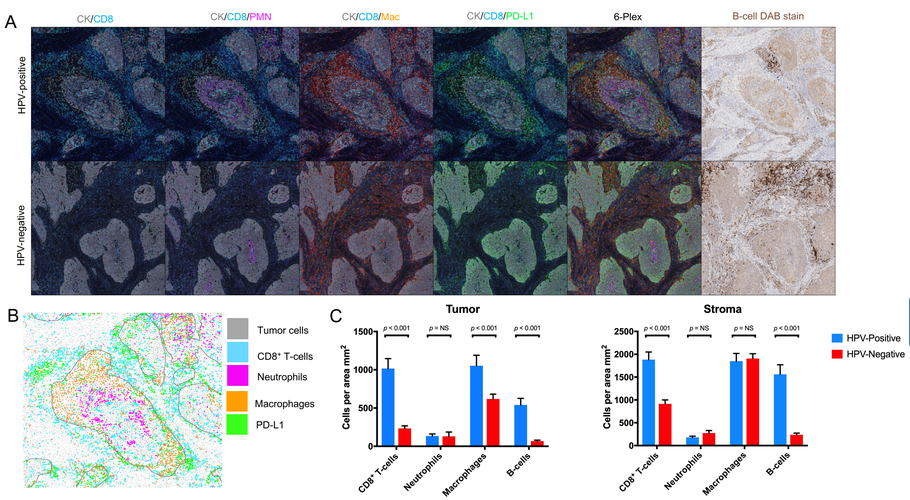

Figure 2: Quantification of spatial relationships of immune cell populations in the HNSCC tumor microenvironment. (A) Representative images displaying sequential IHC of markers of interest along with post-IHC DAB stain for CD19/CD20. (B) HALO spatial plot illustrating characteristic cellular distribution patterns. (C) Cell contents in the tumor and stroma regions of the tumor microenvironment had significant differences in HPV-positive and HPV-negative HNSCC.

RESULTS

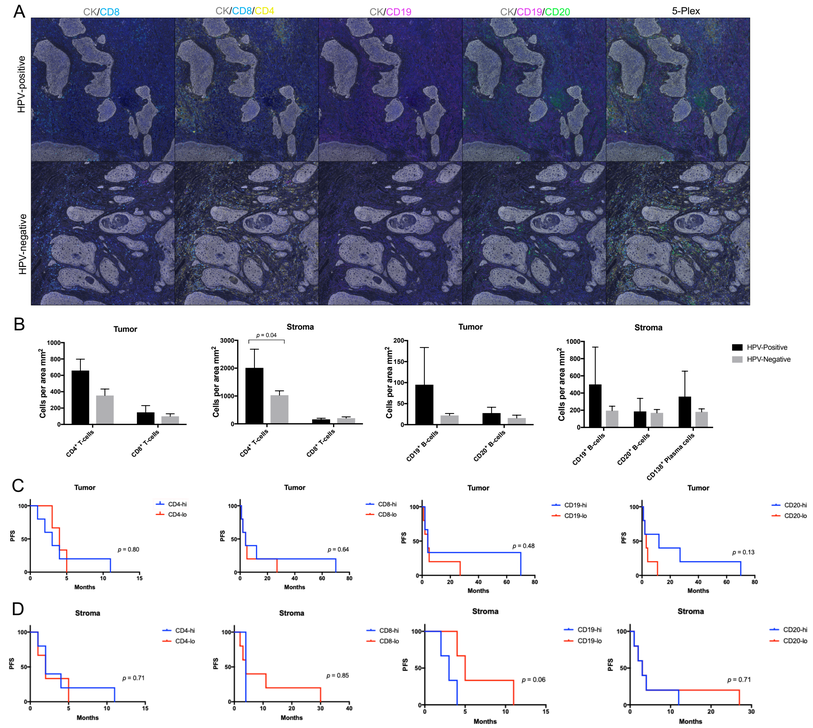

Figure 3: Impact of immune cell populations on HNSCC ICI treatment outcomes. (A) Representative images displaying sequential IHC of markers of interest. (B) Immune cell contents in the tumor and stroma regions of the TME for indicated populations stratified by HPV-status. (C) Kaplan-Meier progression-free survival curves for the lowest vs. the highest quartiles of CD4+ T cells, CD8+ T cells, CD19+ B cells, and CD20+ B cells within the tumor region for HPV-negative HNSCC patients undergoing ICI therapy. (D) Kaplan-Meier progression-free survival curves for the lowest vs. the highest quartiles of CD4+ T cells, CD8+ T cells, CD19+ B cells, CD20+ B cells, and CD138+ plasma cells within the stroma region for HPV-negative HNSCC patients undergoing ICI therapy.

CONCLUSIONS

HPV+ HNSCC displays a B cell and T cell dominant gene expression profile with increased infiltration of these cells in the tumor region compared to HPV- HNSCC. There is significantly less infiltration of these lymphoid cell types in the HPV- HNSCC TME. The B cell signature in HPV+ HNSCC is particularly of interest, given that B cells typically play a major role in resolving viral infections. Higher B cell content trended towards improved PFS in HNSCC patients treated with ICI therapy, but further studies with a larger HNSCC ICI treatment cohort are required to validate the findings presented here.

Contact: Hannan Qureshi, MD
hannanaq@uw.edu