Deciphering Head and Neck Cancer Microenvironment:
Single-cell and Spatial Transcriptomics Reveals Human Papillomavirus-Associated Differences

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Abstract

Background: Human papillomavirus (HPV) is a major causative factor of head and neck squamous cell carcinoma (HNSCC), and the incidence of HPV-associated HNSCC is increasing. We studied the molecular characteristics of primary tumors (PTs) and lymph node metastatic tumors (LNMTs) with stratifying HPV infection.

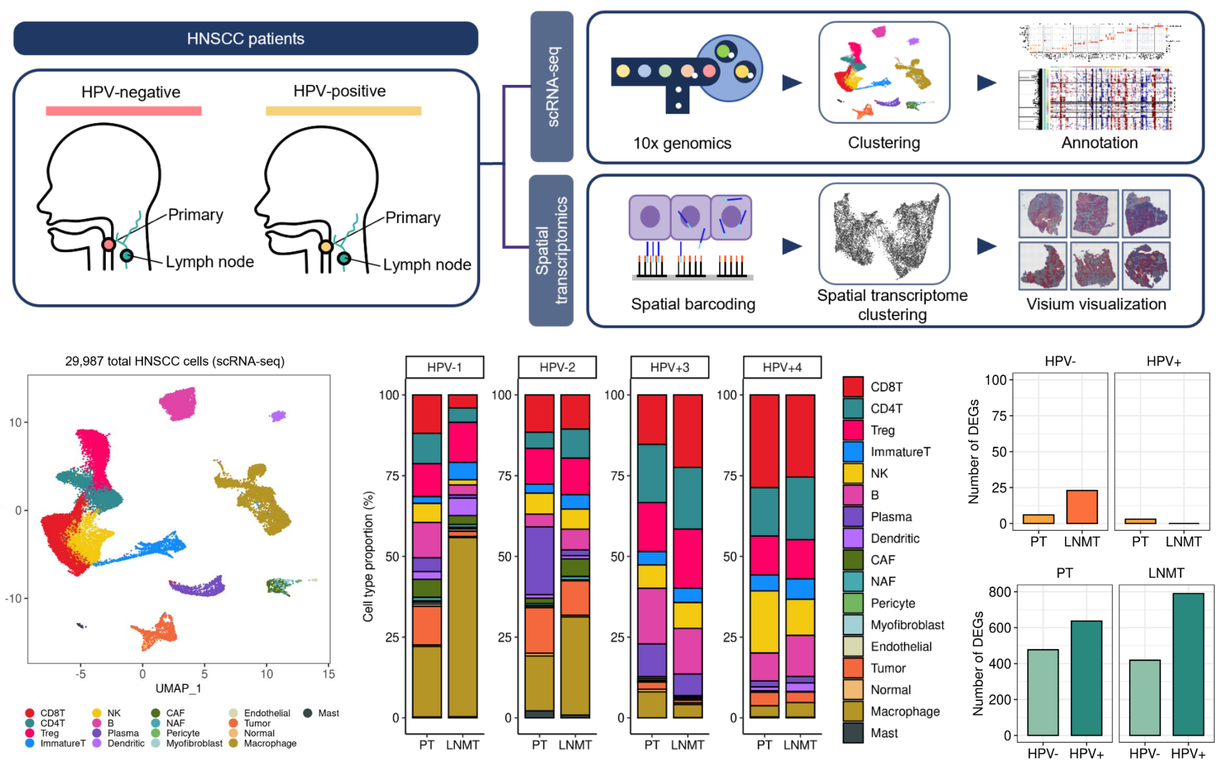
Methods: Eight samples for single-cell RNA profiling and six samples for spatial transcriptomics (ST), composed of matched primary tumors (PT) and lymph node metastases (LNMT), were collected from both HPV-negative (HPV−) and HPV-positive (HPV+) patients. Intracellular and intercellular alterations were analyzed, and the findings were confirmed using experimental validation and publicly available dataset.

Results: The HPV+ tissues were composed of a substantial amount of lymphoid cells regardless of the metastasis, whereas the HPV− tissue exhibited remarkable changes in the number of macrophages and plasma cells, particularly in the LNMT. From both scRNA and ST dataset, we discovered a central gene, pyruvate kinase muscle isoform 2 (PKM2), which is closely associated with the stemness of cancer stem cell (CSC)-like populations in LNMT of HPV− tissue. The consistent expression was observed in HPV− HNSCC cell line and the knockdown of PKM2 weakened spheroid formation ability. We found an ectopic lymphoid structure and clinical effects of the structure in ST slide of the HPV+ patients. We verified their presence in tumor tissue using immunohistochemistry. Finally, the ephrin-A (EPHA2) pathway was detected as important signals in angiogenesis, which is essential for metastasis. The expression of EPHA2 pathway associated genes were also upregulated in HPV− patients ST profiles and confirmed the knockdown of EPHA2 declined the cell migration.

Conclusions: Our study described the distinct cellular composition and molecular alterations in primary and metastatic sites of HNSCC patients based on their HPV status. These results provide insights into HNSCC biology in the context of HPV infection and its potential clinical implications.

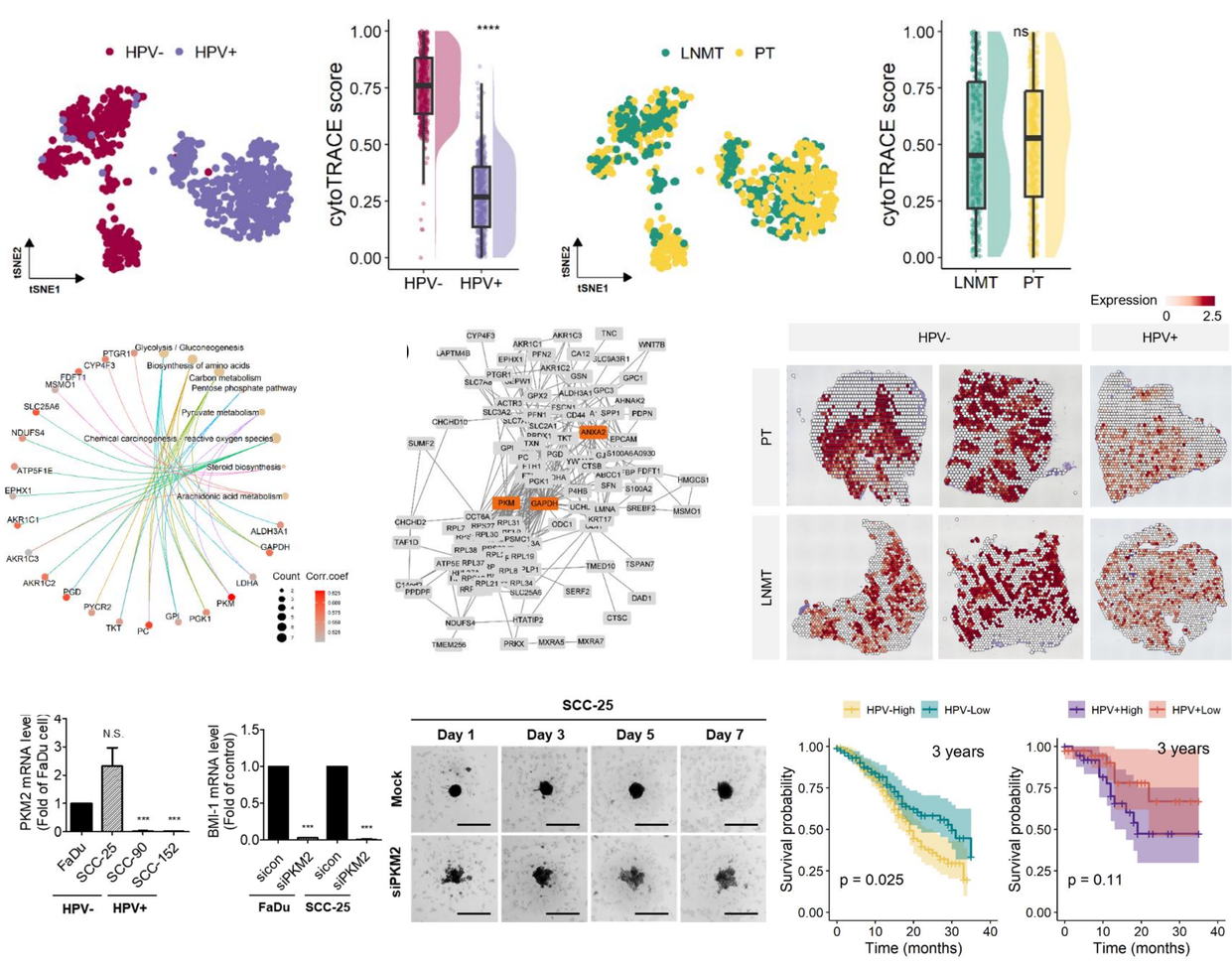
Results & Conclusions

Result 1. Cellular profiles of HNSCC according to HPV infection and tumor site



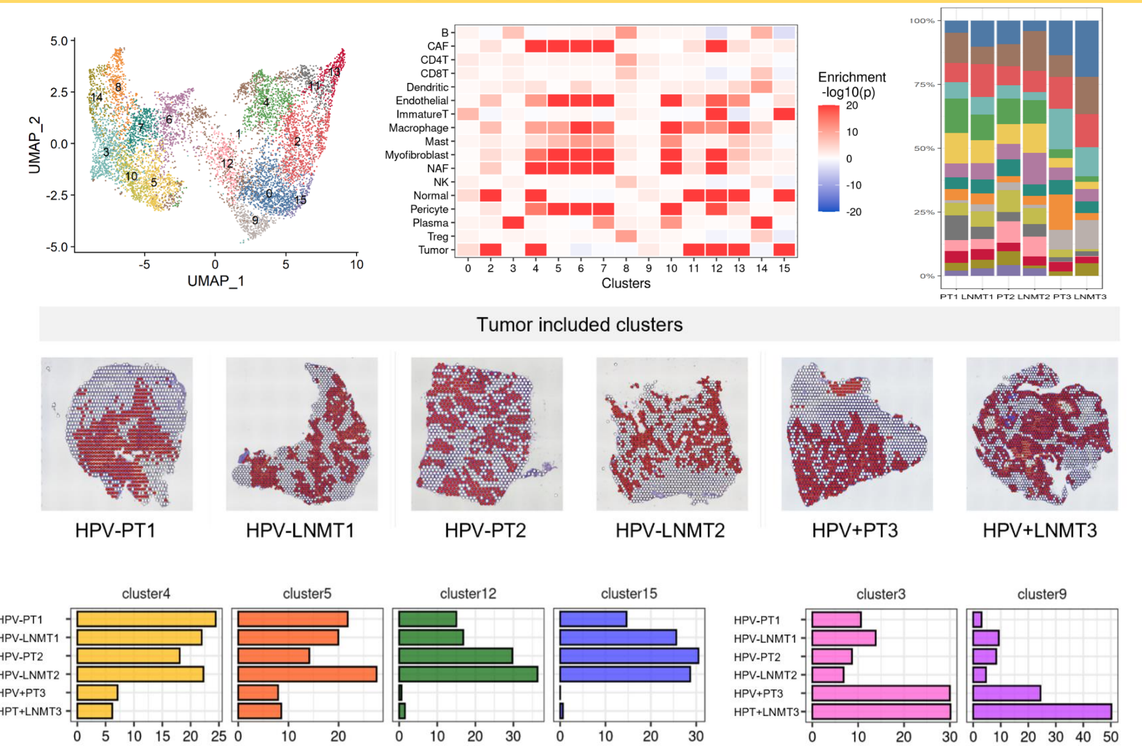
Patients with HNSCC exhibited immunologically different cellular compositions depending on HPV infection status and tumor site. Cancer cells of HNSCC showed distinct characteristics by HPV status rather than by their location.

Result 2. CSC-like population and a highly correlated hub gene, PKM2



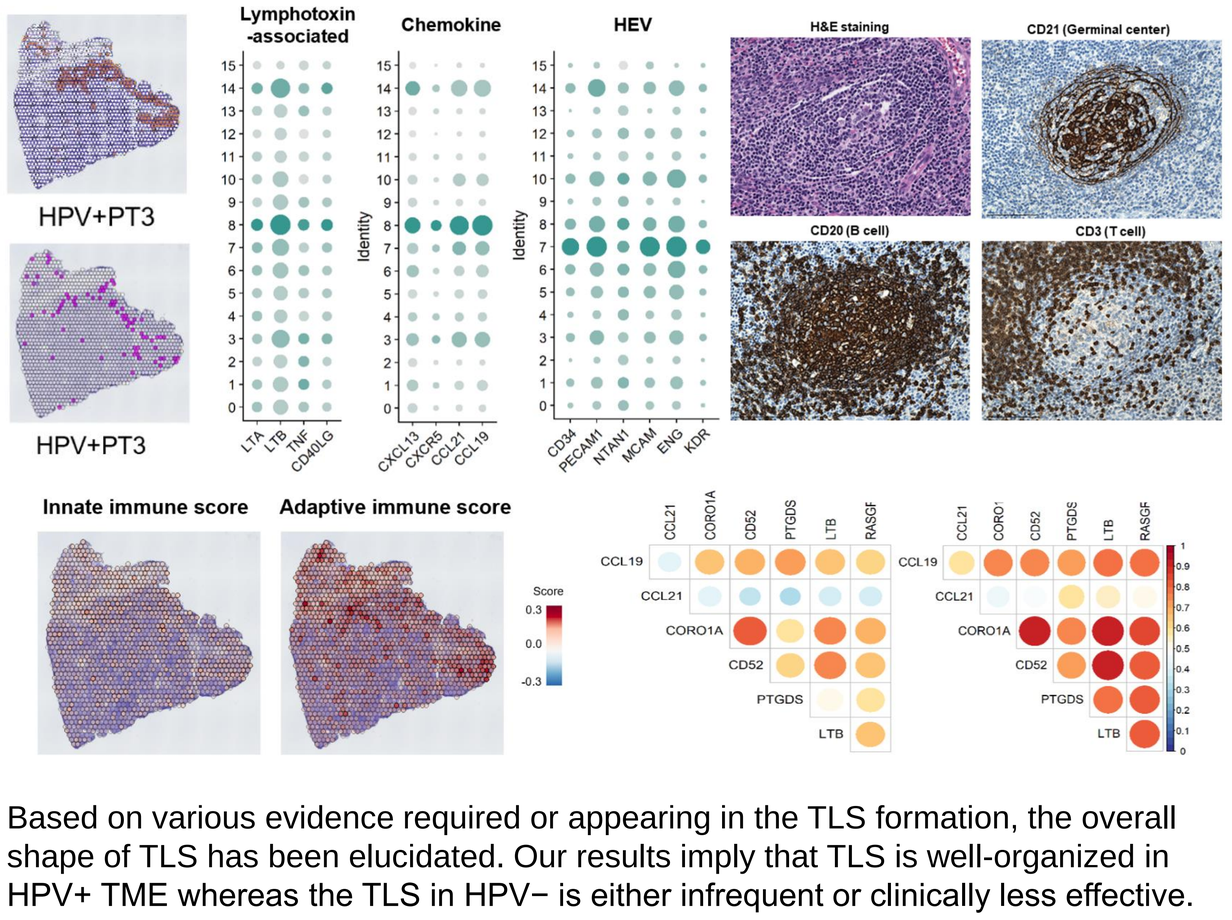
The CSC-like population of HPV− LNMT displayed the highest stemness. We identified a gene with a strong relationship with stemness, PKM2. The PKM2 was responsible to energy production and supported the HPV− tumor progression.

Result 3. Spatial maps of HNSCC tissues



The spot clusters demonstrated a distinct distribution according to HPV infection status. The immune cells actively participate in the development of HPV+ HNSCC, while HPV− HNSCC is characterized as an immune desert.

Result 4. Tertiary lymphoid structures as contributors to the immune response



Based on various evidence required or appearing in the TLS formation, the overall shape of TLS has been elucidated. Our results imply that TLS is well-organized in HPV+ TME whereas the TLS in HPV− is either infrequent or clinically less effective.

Conclusion: In this study, we conducted a transcriptome profiling of PT and matched LNMT sites to investigate their representative properties according to HPV status in HNSCC. Differences were observed in the cellular composition ratio and degree of cancer cell stemness depending on the HPV status, as well as the attributes of the tumor cells. Collectively, we provided comprehensive insights into the heterogeneity of the intratumoral and metastatic environments in HNSCC.