Research Article

The Relationship Between CD8$^+$ T Cells and HPV-Positive Head and Neck Cancer

Diya Sammanna[1], Ashley N. Pearson[2]

[1]*Monta Vista High School, 21840 McClellan Rd, Cupertino, CA, 95014, USA;*
[2]*University of Michigan - Ann Arbor, 500 S State St, Ann Arbor, MI, 48109, USA*

ABSTRACT

HPV-specific head and neck cancer (HNSCC) develops secondary to infection with the human papillomavirus (HPV). HNSCC mostly affects younger and older age groups who live in North America and Europe. T cells play a crucial role in identifying and killing cancer cells in an antigen-specific manner, but cancer cells often evade this natural immunologic detection system. Importantly, the impact of antigen-specific differentiation of T cells in HNSCC is unknown. In this paper, we hypothesized that the location of the tumor and expression of the marker PD-1 is associated with stronger T cell effector functions and T cell activation. To test this hypothesis, we analyzed single cell RNA sequencing (scRNA-seq) for expression of gene programs associated with immunosuppression and T cell effector functions. We found that the lymph node had an increased expression of immunosuppressive genes and that PD-1$^+$ T cells had increased expression of genes associated with effector functions. This work suggests that researchers should focus on improving T cell activation in the lymph node to enhance the anti-tumor immune response in HNSCC.

Keywords: HNSCC, HPV, T cells, PD-1, Lymph node, Tumor

INTRODUCTION

Cancer is a disease that occurs when cells continue to divide and grow due to genetic mutations. Cancer cells do this by evading cell cycle checkpoints that properly regulate when a cell should divide. When many cancer cells group together, these cancer cells can form tumors which can spread through the bloodstream and continue growing in other locations (1, 2). In particular, HPV-specific head and neck cancer (HNSCC) is cancer in the head and neck caused by the human papillomavirus (HPV) that develops in the oropharynx, mostly found in younger individuals and those over 70. This type of cancer is mostly present in people in North America and Europe and who do not breathe or smoke tobacco (3). People with HNSCC have approximately a 50% overall survival rate (4).

T cells are part of the immune system and recognize cancer cells. However, cancer can evade the immune system through methods such as T cell exhaustion (5). Cytotoxic (CD8+) T cells originate from the bone marrow as hematopoietic stem cells that have the potential to become different types of blood cells (6).

The hematopoietic stem cells differentiate into lymphoid progenitors, and into T cell precursors,

Corresponding author: Diya Sammanna, E-mail: diya.sammanna@gmail.com.

Received September 14, 2024**; Accepted** October 22, 2024
https://doi.org/10.70251/HYJR2348.23186195

which then move towards the thymus gland. If the T cell recognizes and binds to its antigen-bound MHC class I molecules, CD8 expression increases and the T cell will differentiate into a mature, cytotoxic effector cell. Exposure to antigen-presenting dendritic cells and inflammatory signaling molecules such as IL2, the T cell is able to identify antigens through parts of pathogen peptides attached to MHC molecules and continue to exert its cytolytic effector function (7). A specific T cell receptor can identify an antigen on the surface of B cells, macrophages, or dendritic cells (8). Then, the B cells, macrophages, or dendritic cells proceed with T cell activation via costimulation, releasing granzymes and perforin (9, 10).

Granzymes are proteases that can break bonds and perforin is a protein that can induce pores, which ultimately lead to apoptosis of the "foreign cell" and prevents the spread of infection (11). The perforin released by the T cell can interact with the BID protein which makes the mitochondria porous, releasing cytochrome C. Cytochrome C and APAF 1 both activate caspase 3, a protein associated with apoptosis. Caspase 3 is able to activate other proteases and break the DNA of the "foreign cell", which ultimately leads to the death of the "foreign cell" (12). The T cell also releases the fas ligand which when bound to the fas receptor, activates caspase 8. Caspase 8 activates caspase 3, which ultimately triggers apoptosis (13).

T cells can be used in anti-tumor immunity. For example, through genetic engineering, T cells can be engineered to synthesize chimeric antigen receptors which attach to cancer cells (14). Antigens are important because they enable the immune system to identify foreign cells and stop the spread of infections. Additionally, antigens allow for the production of vaccines to minimize the effects of diseases (15). However, not much information is known about antigen-specific differentiation of T cells in cancer. Learning more about antigen-specific differentiation could allow for T cells to kill specific cancer cells by recognizing the cancer cell's antigen (16).

Here, the goal was to better understand antigen-specific T cell differentiation in cancer. In this paper, we hypothesized that more effective CD8 T cell activation and resulting effector functions were associated with the tumor and expression of the marker PD1.

MATERIALS AND METHODS

We analyzed 18 treatment-naive tissue samples from 5 patients with HPV+ head and neck cancer (accession: GSE180268). The dataset contained single cell RNA sequencing results of $CD8^+$ T cells isolated from lymph node and tumor. The patients (IDs 7, 15, 34, 37, 51) had not received treatment before for HPV positive head and neck cancer. The samples were collected at different locations, specifically the lymph node and the tumor, to compare the differences between the $CD8^+$ T cells at each location. The samples expressed markers KSA, PD1, or QVD; TILs were taken from the tumor and lymph node and stained with a tetramer (17).

To address this issue, researchers worked with 12 patients with HPV positive head and neck cancer to take samples of 6 primary tumors and 8 metastatic lymph nodes with different markers (17). To analyze and compare TILs, single cell RNA sequencing was performed on HPV-specific PD-1+ $CD8^+$ TILs that were sorted based on which marker they expressed. In the tumor of patients diagnosed with HPV-positive HNSCC, functional HPV-specific PD-1+TCF-1+$CD8^+$ T cells were present; functional HPV-specific PD-1+TCF-1+$CD8^+$ T cells were shown to improve T cell function after PD-1 blockade and are associated with an improvement to PD-1 therapy. Additionally, cancer specific therapeutic HPV vaccines usually focus on the E6 and E7 antigens, but also targeting the E2 and E5 antigens could specifically strongly activate $CD8^+$ T cells, which could prevent the formation of tumors. Furthermore, research around $CD8^+$ T cells and B cells with HPV-positive HNSCC can help give more insight on other types of cancers caused by diseases.

We analyzed the data set to identify the effect of HPV-specific PD-1+ stem-like CD8 T cells in head and neck cancer. This was done through scRNA-seq using RStudio and the following packages: tidyverse, Seurat, pheatmap, RColorBrewer, scales, cowplot, patchwork, grid, gridExtra, harmony, and ggplot2.

Principal Component Analysis (PCA) is a statistical technique used to reduce the number of dimensions in a data set to improve visualization and understanding (Figure 1A). PCA creates Principal Components (PC) and a few of these PCs (PC1, PC2) capture the greatest variance in the data (PC1 accounts for more variance than PC2). Elbow plots are used to determine the percentage of variance in the data each PC captures. PCA allows for easier identification of patterns in the data because of the simplification into principal components which capture the most important parts of the data (18).

Uniform Manifold Approximation and Projection (UMAP) is a statistical technique used to allow for easier graphing of complicated data (Figure 1B). The computer

calculates and plots the distance between the data points linearly on the x-axis. Then, the computer draws a curve relative to these points to calculate similarity scores. The data is plotted on a two-dimensional graph based on similarity scores to form clusters. Data points that are in the same cluster are closely related but the amount of difference between different clusters cannot be calculated based on the distance on the graph (19).

We named the UMAP clusters using feature plots and violin plots. We named clusters 0, 1, 3, 7, and 8 TRM cells using the genes *IFITM1, LGALS3, ITGB1, VIM,* and *CRIP1* (Figure 2A); cluster 2 inflammatory cells using the genes *CCL5, GZMK, and ITGA4* (Figure 2B); clusters 4 and 5 naive cells using the genes *CCR7, LEF1, S1PR1, CF7,* and *SELL* (Figure 2C); and cluster 6 terminal effector cells using the genes *ID2, CXCR6, CCL4, IFNG, GZMB, PRF1, PDCD1* (Figure 2D) (20, 21, 22, 23).

RESULTS

We wanted to understand the differences in T cell diversity compared between patients, between locations, and between tetramers. To do so, we created UMAPs (Figure 2E) and bar graphs (Figure 3) split by patient ID, location, and tetramer.

We observed that the lymph node had fewer

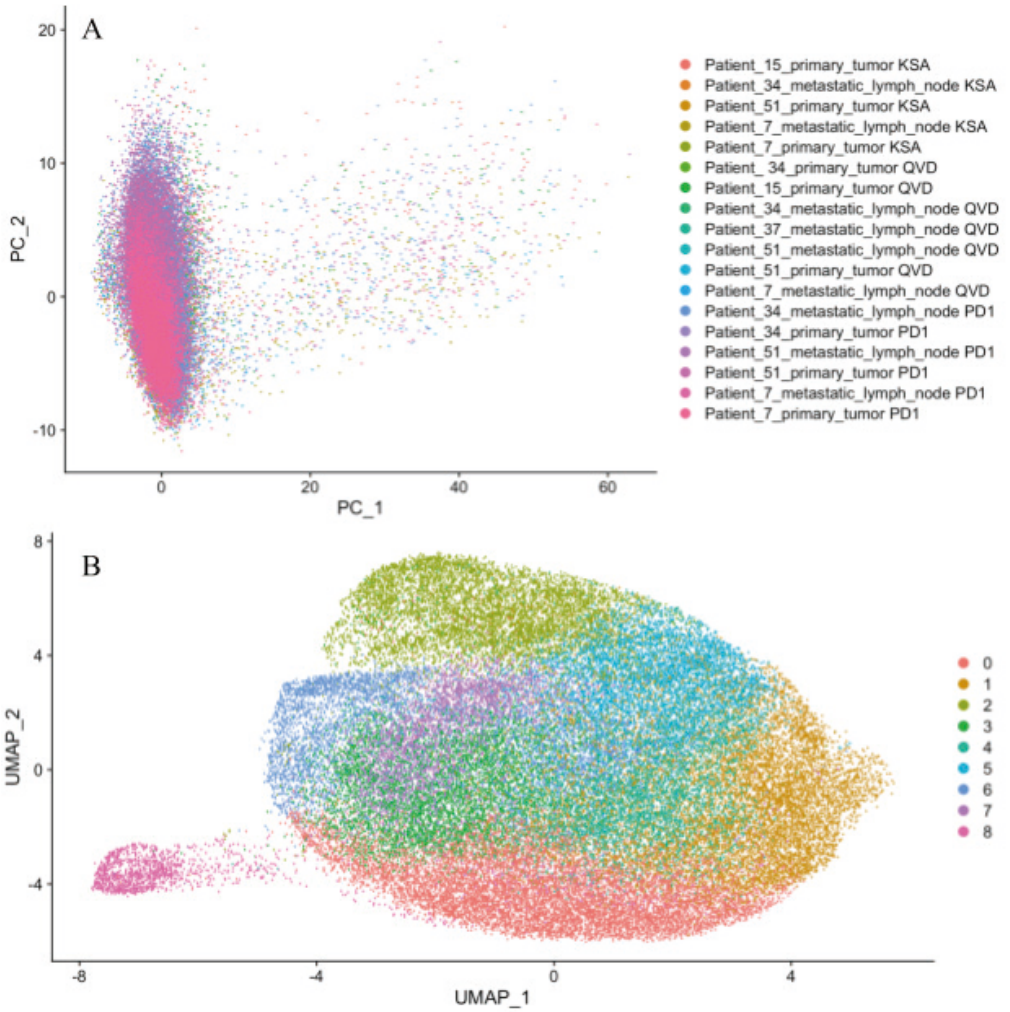

Figure 1. 18 samples of T cells from the lymph node and the tumor A) PCA plot of TILs with varying patients, locations, and tetramer B) UMAP of TILs with 8 T cell clusters after removal of Cluster 9.

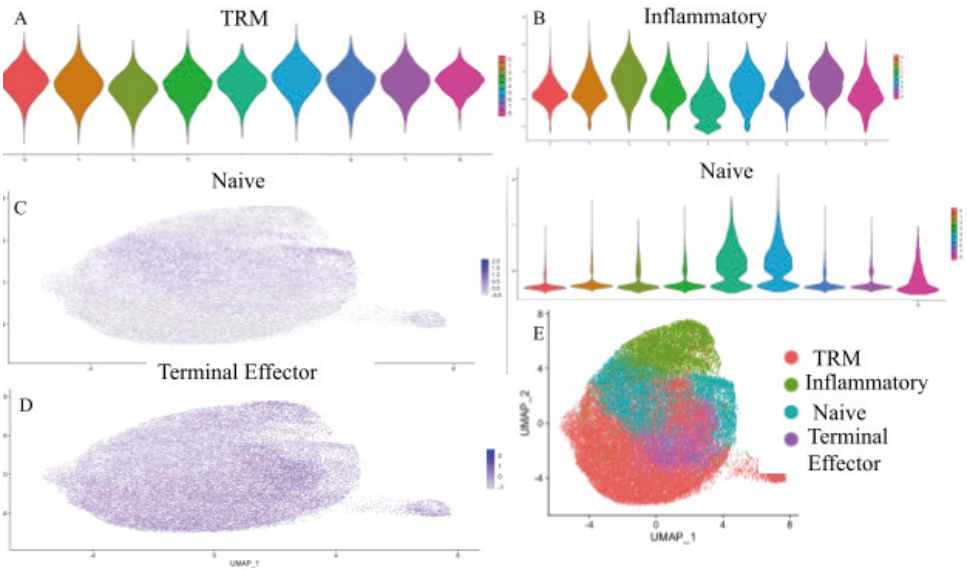

Figure 2. Strategies used to name UMAP clusters. A) Violin Plot of TRM Cells using the Genes *IFITM1, LGALS3, ITGB1, VIM,* and *CRIP1* B) Violin Plot of Inflammatory Cells using the Genes *CCL5, GZMK,* and *ITGA4*. C) Feature Plot and Violin Plot of Naive Cells using the Genes *CCR7, LEF1, S1PR1, CF7,* and *SELL* D) Feature Plot of Terminal Effector Cells using the Genes *ID2, CXCR6, CCL4, IFNG, GZMB, PRF1, PDCD1* E) UMAP of Named Clusters.

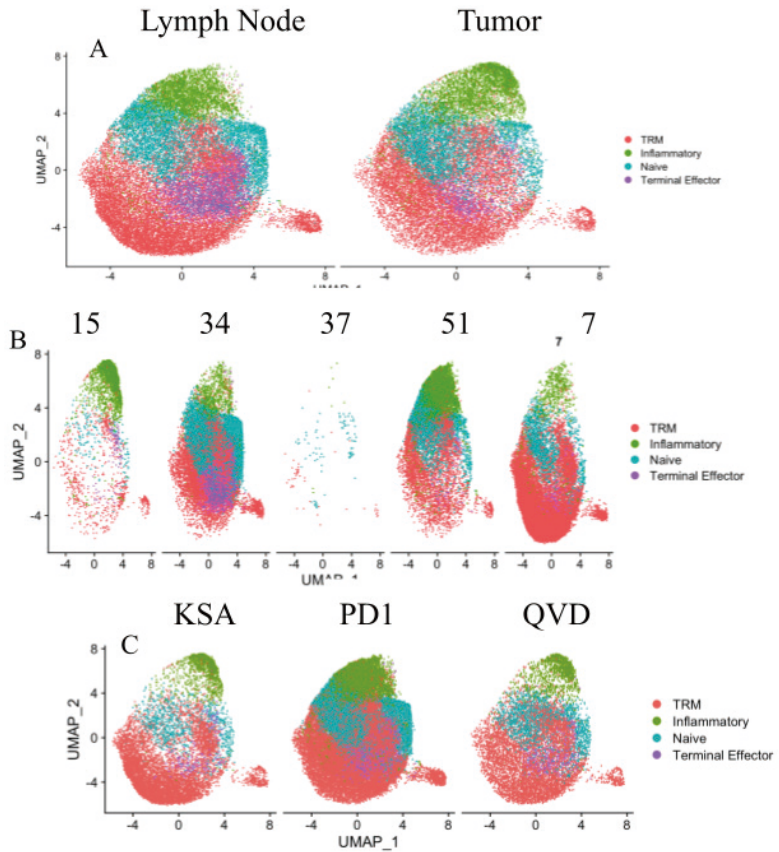

Figure 3. T cell diversity varies based on location, patient, and tetramer. A) UMAP of T cells split by Location B) UMAP of T cells split by Patient C) UMAP of T cells split by Tetramer.

inflammatory cells than the tumor (Figure 4A). We hypothesized that if the lymph node has a smaller proportion of inflammatory cells, then the lymph node would have less T cell activation and more T cell immunosuppression. To test this, we created violin plots and feature plots split by location to measure the expression level of immunosuppression T cell genes *CTLA4* (Figure 5A), *PDCD1* (Figure 5B), *CD247* (Figure 5C), and *ICOS* (Figure 5D) (24, 25, 26, 27).

We found that *CTLA4, PDCD1, CD247,* and *ICOS* are expressed more in the lymph node than in the tumor. This means T cells in the lymph node are blocked from being activated. Immunosuppression has the effects of altering Myc, mTOR, AMPK, and H1F1α which control T cell reactions. Additionally, immunosuppression changes glycolysis and glutaminolysis which plays a role in the citric acid cycle (28, 29).

We observed that patients that expressed the marker

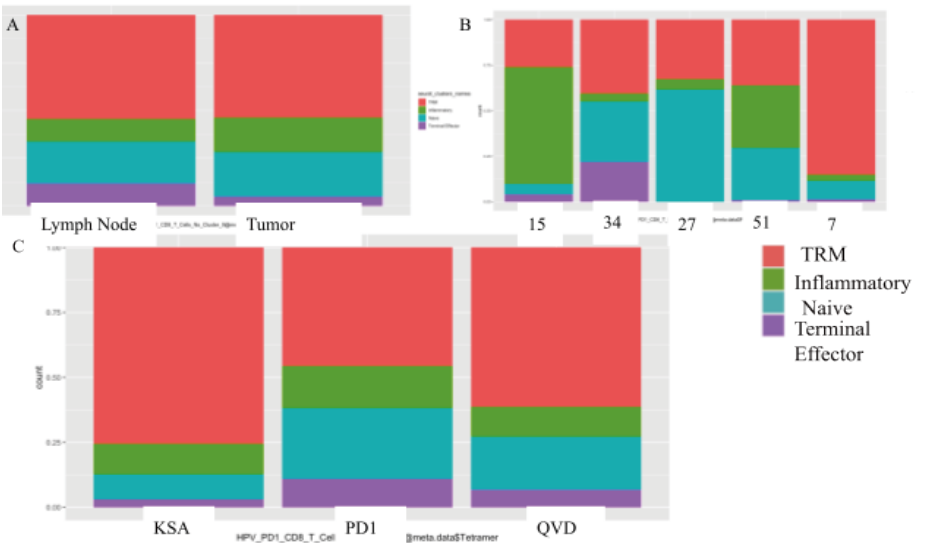

Figure 4. Stacked Bar Graphs of T cells split by Location, Patient, and Tetramer. A) Stacked bar graph of T cells split by location B) Stacked bar graph of T cells split by Patient C) Stacked bar graph of T cells split by Tetramer.

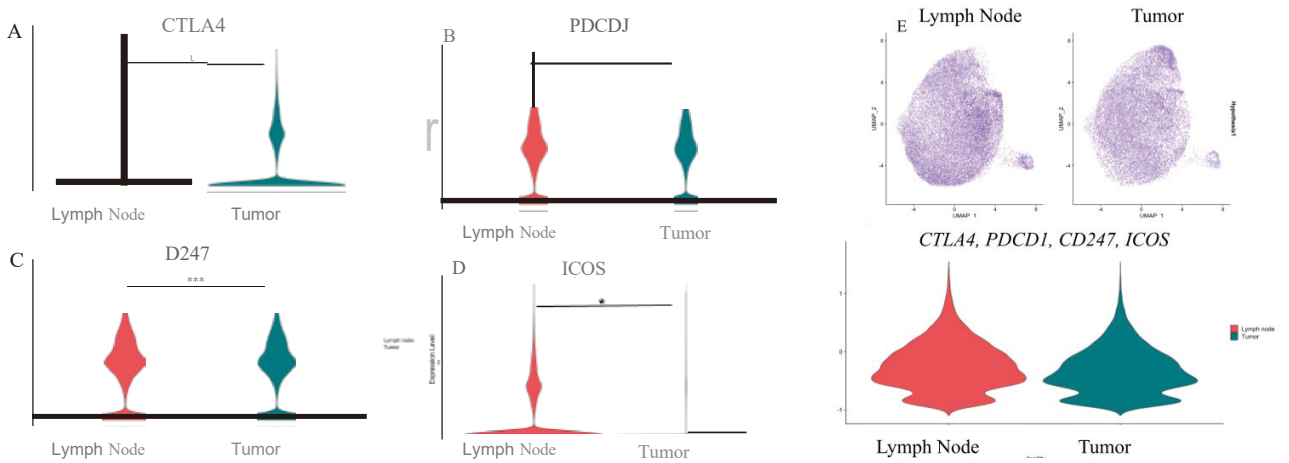

Figure 5. The lymph node has increased expression of immunosuppressive genes compared to the tumor. A) Violin plot of expression of the gene *CTLA4* in the lymph node and the tumor. B) Violin plot of expression of the gene *PDCD1* in the lymph node and the tumor. C) Violin plot of expression of the gene *CD247* in the lymph node and the tumor. D) Violin plot of expression of the gene *ICOS* in the lymph node and the tumor. E) Feature plot (top) and Violin plot (bottom) of the expression of the genes *CTLA4, PDCD1, CD247,* and *ICOS* in the lymph node and the tumor.

PD1 have a higher proportion of terminal effector cells than patients that expressed the markers QVD or KSA. We hypothesized that if T cells that expressed the marker PD1 have a higher proportion of terminal effector cells, then T cells that expressed marker PD1 would have more effector functions. To test this, we created violin and feature plots split by tetramers to measure the expression level of genes associated with T cell effector functions, including *GZMK* (Figure 6A), *CCR7* (Figure 6B), *CCL5* (Figure 6C) and *GZMM* (Figure 6D) (30, 31, 32). We found that *GZMK, CCR7, CCL5,* and *GZMM* are expressed higher in patients that expressed the marker PD1 compared to patients that expressed the markers KSA or QVD. This signifies that there are more effector functions in T cells that expressed the marker PD1 compared to KSA or QVD.

We observed that Patient 15 has a smaller proportion of naive T cells compared to Patients 7, 34, 37, and 51. We hypothesized that if Patient 15 has a smaller proportion of naive cells, then Patient 15 would have more T cell activation. To test this, we created violin and feature plots split by patient to measure the expression of T cell activation genes *IRF7* (Figure 7A), *GZMK* (Figure 7B), *EOMES* (Figure 7C), *ZEB2* (Figure 7D), and *NKG7* (Figure 7E) (33). We found that Patient 15 had a higher expression of genes *IRF7, GZMK, EOMES, ZEB2* and *NKG7*. This means Patient 15 has more overall T cell activation.

DISCUSSION

In this paper, we found that the lymph node has fewer inflammatory cells compared to the tumor (Figure 4A), and that there is a higher expression of immunosuppressive genes in the lymph node (Figure 5A, 5B, 5C, and 5D), indicating that T cells in the lymph node might be blocked from activation. We also found that there was a higher proportion of terminal effector cells in patients that expressed the marker PD1 compared to the markers KSA or QVD (Figure 4C) and a higher amount of gene expression associated with effector functions in patients that expressed the marker PD1 (Figure 6A, 6B, 6C, and 6D).

Additionally, Patient 15 has a smaller proportion of naive cells compared to Patients 7, 34, 37, and 51 (Figure 4B) and Patient 15 has a higher expression of T cell activation genes (Figure 7A, 7B, 7C, 7D, and 7E).

T cell effector functions have an important role in the anti-tumor response. Effector T cells are able to kill cancer cells (34). These results indicate that the location of the tumor, expressing the marker PD1, and Patient 15 have a higher amount of T cell activation and effector functions such as releasing cytokines to kill cancer cells (35). These results agree with the original study as Eberhardt, Kissick, and researchers found that PD-1+ stem-like $CD8^+$ T cells

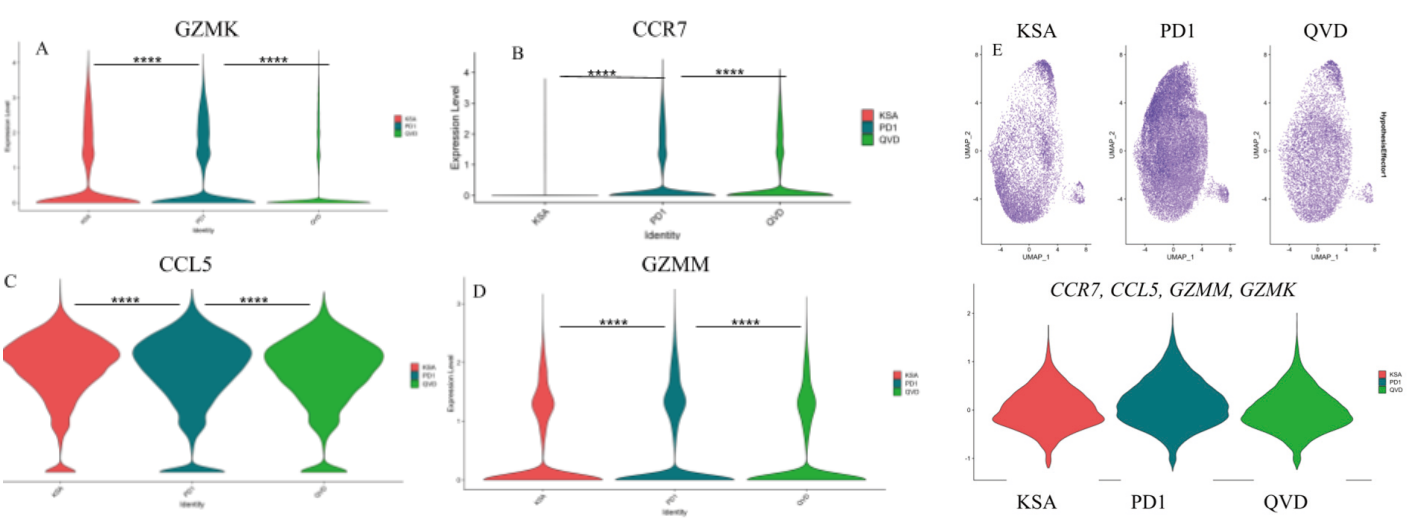

Figure 6. Tetramer PD1 has increased expression of genes associated with T cell effector functions. A) Violin plot of expression of the gene *GZMK* in the markers PD1, QVD, and KSA. B) Violin plot of expression of the gene *CCR7* in the markers PD1, QVD, and KSA. C) Violin plot of expression of the gene *CCL5* in the markers PD1, QVD, and KSA. D) Violin plot of expression of the gene *GZMM* in the markers PD1, QVD, and KSA. E) Feature plot (top) and violin plot (bottom) of the expression of the genes *GZMK,CCR7,CCL5,* and *GZMM* in the markers PD1, QVD, and KSA.

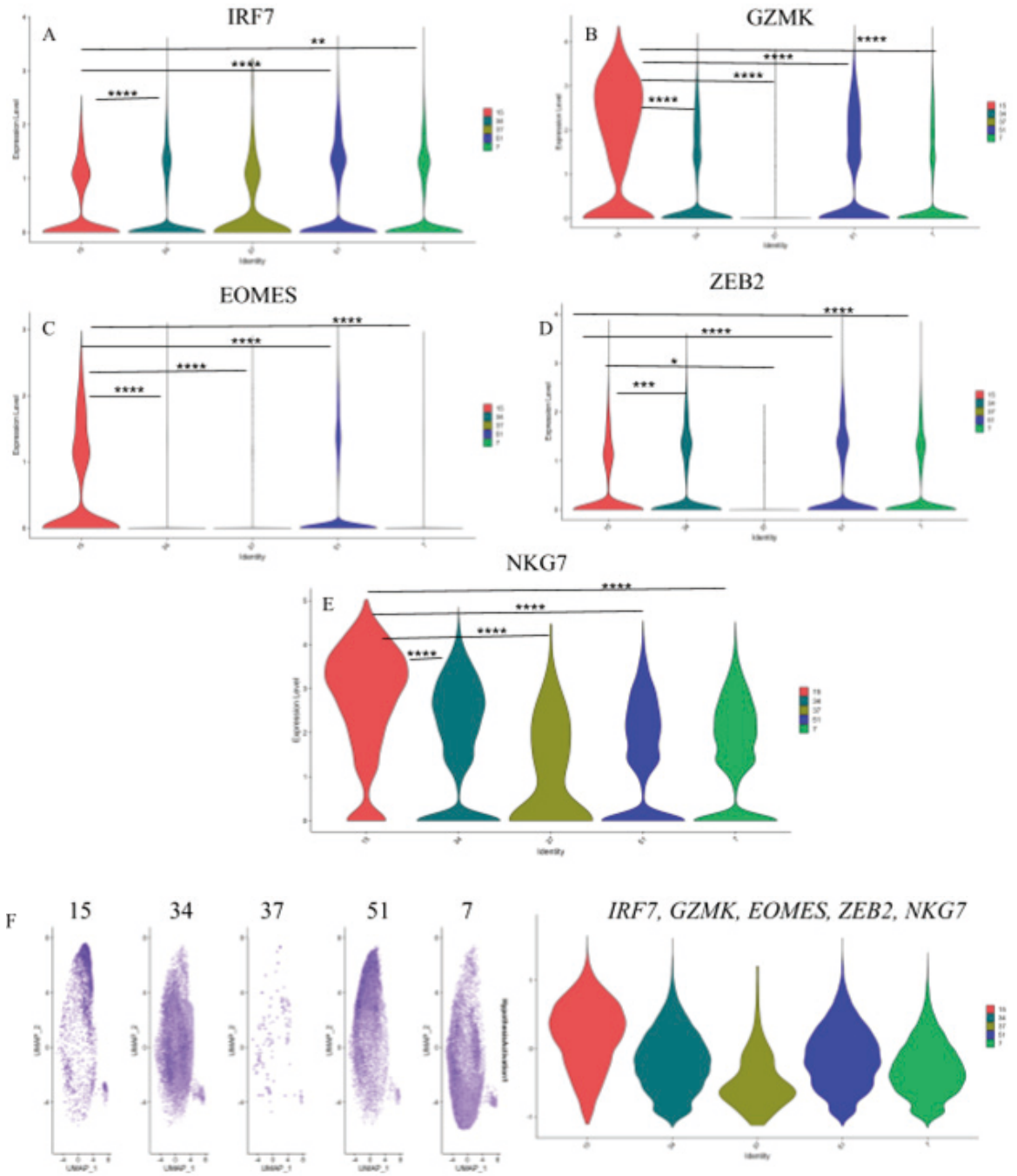

Figure 7. Patient 15 has increased expression of T cell activation genes. A) Violin plot of expression of the gene *IRF7* in Patients 7, 15, 34, 37, and 51 B) Violin plot of expression of the gene *GZMK* in Patients 7, 15, 34, 37, and 51 C) Violin plot of expression of the gene *EOMES* in Patients 7, 15, 34, 37, and 51 D) Violin plot of expression of the gene *ZEB2* in Patients 7, 15, 34, 37, and 51. E) Violin plot of expression of the gene *NKG7* in Patients 7, 15, 34, 37, and 51. F) Feature plot (left) and violin plot (right) of the expression of the genes *IRF7, GZMK, EOMES, ZEB2,* and *NKG7* in Patients 7, 15, 34, 37, and 51.

are associated with stronger T cell reactions in cases with sustained antigens (17). In our analysis, we found that samples that expressed the PD-1 marker had more T cell effector functions. Additionally, cancer and other viruses cause the PD1 receptor to be blocked which causes a decrease in $CD8^+$ T cell activity. However, reversing this blockade of the PD1 receptors has been found to improve T cell activity (17).

One of the limitations of this study and the original analysis is the amount of data available for Patient 37. Patient 37 had a small amount of TILs that were sequenced. Additionally, this study used one published data set. However, this research uses a variety of visual and statistical techniques and creates original hypotheses.

Future research should focus on how to improve T cell activation and the anti-tumor response, specifically in the lymph node, as the lymph node had more expression of immunosuppressive genes and less T cell activation. Increasing T cell activation, a part of immunotherapy, is a promising method to reduce the number of cancer cells. Cancer immunotherapy specifically uses various kinds of T cells to target the antigens on the surface of cancer cells. Cancer cells attach to PD-1 and CTLA-4 which induce immunosuppression on T cells. Using methods to reverse the PD-1 and CTLA-4 blockade could potentially increase T cell activation, and the immune response to kill cancer cells. One example of this is a type of molecular therapy, immune checkpoint inhibitors (ICI), which deregulate the expression of PD-1, CTLA-4, and PD-1 (36).

Another example of targeting T cell inhibitors is applying chemotherapy and immune checkpoint blockade (ICB) (37). ICBs are used to help with the blockade of receptors and are used to strengthen the immune system response to chemotherapy. Chemotherapy is the process of removing cancer cells through using chemicals (38). Combining these two methods could help enhance the anti-tumor response in the lymph node (37).

CONCLUSION

HNSCC is a cancer which develops secondary to HPV infection. This study used 18 samples from 5 patients with HPV+ head and neck cancer. We identified that $PD\text{-}1^+$ T cells had an increased expression of genes linked with T cell effector functions and there was also an increased expression of immunosuppressive genes in the lymph node. This research shows that improving T cell activation is an important part of killing cancer cells. In the future, researchers should focus on decreasing the expression of PD-1, CTLA-4, and PD-1 to increase T cell activation. For example, researchers could use ICIs or both ICBs and chemotherapy to deregulate the expression of these proteins.

ACKNOWLEDGEMENTS

I would like to acknowledge my mentors Ashley N. Pearson and Dr. Michael Martinez for providing me with guidance for this project.

DECLARATION OF CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Foster I. Cancer: A cell cycle defect. Radiography. 2008; 14 (2): 144-149. https://doi.org/10.1016/j.radi.2006.12.001
2. Nicolson GL. Cancer Metastasis. Scientific American. 1979; 240 (3): 66-77. http://www.jstor.org/stable/24965152. https://doi.org/10.1038/scientificamerican0379-66
3. Sabatini ME & Chiocca S. Human papillomavirus as a driver of head and neck cancers. British journal of cancer. 2020; 122 (3): 306-314. https://doi.org/10.1038/s41416-019-0602-7
4. Šimić I, Božinović K, Milutin Gašperov N, Kordić M, Pešut E, Manojlović L, ... & Sabol I. Head and Neck Cancer Patients' Survival According to HPV Status, miRNA Profiling, and Tumour Features-A Cohort Study. International journal of molecular sciences. 2023; 24 (4): 3344. https://doi.org/10.3390/ijms24043344
5. Muenst S, Läubli H, Soysal SD, Zippelius A, Tzankov A & Hoeller S. The immune system and cancer evasion strategies: therapeutic concepts. Journal of internal medicine. 2016; 279 (6): 541-562. https://doi.org/10.1111/joim.12470
6. Schürch CM, Riether C & Ochsenbein AF. Cytotoxic CD8+ T cells stimulate hematopoietic progenitors by promoting cytokine release from bone marrow mesenchymal stromal cells. Cell stem cell. 2014; 14 (4): 460-472. https://doi.org/10.1016/j.stem.2014.01.002
7. Viret C & Janeway Jr, CA. MHC and T cell development. Reviews in immunogenetics. 1999; 1 (1): 91-104.
8. Huppa JB & Davis MM. T-cell-antigen recognition and the immunological synapse. Nature Reviews Immunology. 2003; 3 (12): 973-983. https://doi.org/10.1038/nri1245
9. Sharpe AH. Mechanisms of costimulation. Immunological reviews. 2009; 229 (1): 5. https://doi.org/10.1111/j.1600-065X.2009.00784.x
10. Quann EJ, Merino E, Furuta T & Huse M. Localized diacylglycerol drives the polarization of the microtubule-

organizing center in T cells. Nature immunology. 2009; 10 (6): 627-635. https://doi.org/10.1038/ni.1734
11. Trapani JA & Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nature Reviews Immunology. 2002; 2 (10): 735-747. https://doi.org/10.1038/nri911
12. Janicke RU, Sprengart ML, Wati MR & Porter AG. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. Journal of Biological Chemistry. 1998; 273 (16): 9357-9360. https://doi.org/10.1074/jbc.273.16.9357
13. Stennicke HR, Jurgensmeier JM, Shin H, Deveraux Q, Wolf BB, Yang X, ... & Salvesen GS. Pro-caspase-3 is a major physiologic target of caspase-8. Journal of Biological Chemistry. 1998; 273 (42): 27084-27090. https://doi.org/10.1074/jbc.273.42.27084
14. Zhang C, Liu J, Zhong JF & Zhang X. Engineering car-t cells. Biomarker research. 2017; 5: 1-6. https://doi.org/10.1186/s40364-017-0081-z
15. Devaraj K, Gillison ML & Wu TC. Development of HPV vaccines for HPV-associated head and neck squamous cell carcinoma. Critical Reviews in Oral Biology & Medicine. 2003; 14 (5): 345-362.
16. Zhang Y, Wang N, Ding M, Yang Y, Wang Z, Huang L, ... & Wu S. CD40 accelerates the antigen-specific stem-like memory CD8+ T Cells formation and Human Papilloma Virus (HPV)-positive tumor eradication. Frontiers in Immunology. 2020; 11: 1012. https://doi.org/10.3389/fimmu.2020.01012
17. Eberhardt CS, Kissick HT, Patel MR, Cardenas MA, Prokhnevska N, Obeng RC, ... & Ahmed R. Functional HPV-specific PD-1+ stem-like CD8 T cells in head and neck cancer. Nature. 2021; 597 (7875): 279-284. https://doi.org/10.1038/s41586-021-03862-z
18. Zhuang H, Wang H & Ji Z. findPC: An R package to automatically select the number of principal components in single-cell analysis. Bioinformatics. 2022; 38 (10): 2949-2951. https://doi.org/10.1093/bioinformatics/btac235
19. McInnes L, Healy J & Melville J. (2018). Umap: Uniform manifold approximation and projection for dimension reduction. arXiv preprint arXiv:1802.03426. https://doi.org/10.21105/joss.00861
20. Zhang Q, Jiang L, Wang W, Huber AK, Valvo VM, Jungles KM, ... & Morgan MA. Potentiating the radiation-induced type I interferon antitumoral immune response by ATM inhibition in pancreatic cancer. JCI insight. 2024; 9 (6). https://doi.org/10.1172/jci.insight.168824
21. Mendoza A, Fang V, Chen C, Serasinghe M, Verma A, Muller J, ... & Schwab SR. Lymphatic endothelial S1P promotes mitochondrial function and survival in naive T cells. Nature. 2017; 546 (7656): 158-161. https://doi.org/10.1038/nature22352
22. Ahn E, Araki K, Hashimoto M, Li W, Riley JL, Cheung J, ... & Ahmed R. Role of PD-1 during effector CD8 T cell differentiation. Proceedings of the National Academy of Sciences. 2018; 115 (18): 4749-4754. https://doi.org/10.1073/pnas.1718217115
23. Omilusik KD, Nadjsombati MS, Shaw LA, Yu B, Milner J. J & Goldrath AW. Sustained Id2 regulation of E proteins is required for terminal differentiation of effector CD8+ T cells. Journal of Experimental Medicine. 2018; 215 (3): 773-783. https://doi.org/10.1084/jem.20171584
24. Linsley PS, Wallace PM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, ... & Tepper MA. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science. 1992; 257 (5071): 792-795. https://doi.org/10.1126/science.1496399
25. Röver LK, Gevensleben H, Dietrich J, Bootz F, Landsberg J, Goltz D, & Dietrich D. PD-1 (PDCD1) promoter methylation is a prognostic factor in patients with diffuse lower-grade gliomas harboring isocitrate dehydrogenase (IDH) mutations. EBioMedicine. 2018; 28: 97-104. https://doi.org/10.1016/j.ebiom.2018.01.016
26. Christopoulos P, Dopfer EP, Malkovsky M, Esser PR, Schaefer HE, Marx A, ... & Fisch P. A novel thymoma-associated immunodeficiency with increased naive T cells and reduced CD247 expression. The Journal of Immunology. 2015; 194 (7): 3045-3053. https://doi.org/10.4049/jimmunol.1402805
27. Conrad C, Gregorio J, Wang YH, Ito T, Meller S, Hanabuchi S, ... & Gilliet M. Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3+ T-regulatory cells. Cancer research. 2012; 72 (20): 5240-5249. https://doi.org/10.1158/0008-5472.CAN-12-2271
28. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. Nature reviews immunology. 2012; 12 (5): 325-338. https://doi.org/10.1038/nri3198
29. Pająk B, Zieliński R & Priebe W. The Impact of Glycolysis and Its Inhibitors on the Immune Response to Inflammation and Autoimmunity. Molecules. 2024; 29 (6): 1298. https://doi.org/10.3390/molecules29061298
30. Romero V & Andrade F. Non-apoptotic functions of granzymes. Tissue antigens. 2008; 71 (5): 409-416. https://doi.org/10.1111/j.1399-0039.2008.01013.x
31. Campbell JJ, Murphy KE, Kunkel EJ, Brightling CE, Soler D, Shen Z, ... & Wu L. CCR7 expression and memory T cell diversity in humans. The Journal of Immunology. 2001; 166 (2): 877-884. https://doi.org/10.4049/jimmunol.166.2.877
32. Keophiphath M, Rouault C, Divoux A, Clément K & Lacasa D. CCL5 promotes macrophage recruitment and survival in human adipose tissue. Arteriosclerosis, thrombosis, and vascular biology. 2010; 30 (1): 39-45. https://doi.org/10.1161/ATVBAHA.109.197442
33. Szabo PA, Levitin HM, Miron M, Snyder ME, Senda T, Yuan J, ... & Sims PA. Single-cell transcriptomics of human

T cells reveals tissue and activation signatures in health and disease. Nature communications. 2019; 10 (1): 4706. https://doi.org/10.1038/s41467-019-12464-3

34. Bercovici N & Trautmann A. Revisiting the role of T cells in tumor regression. Oncoimmunology. 2012; 1 (3): 346-350. https://doi.org/10.4161/onci.18800
35. Restifo NP & Gattinoni L. Lineage relationship of effector and memory T cells. Current opinion in immunology. 2013; 25 (5): 556-563. https://doi.org/10.1016/j.coi.2013.09.003
36. Liu M & Guo F. Recent updates on cancer immunotherapy. Precision clinical medicine. 2018; 1 (2): 65-74. https://doi.org/10.1093/pcmedi/pby011
37. Heinhuis KM, Ros W, Kok M, Steeghs N, Beijnen JH, & Schellens JHM. Enhancing antitumor response by combining immune checkpoint inhibitors with chemotherapy in solid tumors. Annals of Oncology. 2019; 30 (2): 219-235. https://doi.org/10.1093/annonc/mdy551
38. Nygren P. What is cancer chemotherapy?. Acta Oncologica. 2001; 40 (2-3): 166-174. https://doi.org/10.1080/02841860151116204