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Review Article

Emerging Trends in the Pathological Research of Human Papillomavirus-positive Oropharyngeal Squamous Cell Carcinoma

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Abstract

Oropharyngeal squamous cell carcinomas (OPSCCs) have shown an alarming rate of increase in incidence over the past several decades, markedly in men. In the United States, transcriptionally-active human papillomavirus (HPV), particularly HPV 16, has become the highest contributive agent of OPSCCs, affecting approximately 16,000 people a year. Compared to patients with HPV-negative OPSCCs, patients with HPV-positive OPSCCs exhibit better health responses to chemoradiotherapy and an overall increase in long-term survival. Despite promising treatment options, many OPSCCs are discovered at an advanced stage, and ~20% of cases will recur after definitive treatment. Therefore, extensive research is ongoing to identify new targets for precision treatment and to stratify tumor prognosis. The aim of this review is to capture the most updated research on HPV-positive OPSCCs, emphasizing their relevance as potential new targets for precision medicine and survival prognosis.

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Introduction

Approximately 80% of oropharyngeal squamous cell carcinomas (OPSCCs), which include oropharyngeal wall, tonsillar, and base-of-tongue tumors, are induced by high-risk human papillomavirus (HPV) (Fig. 1).[1–3] HPV-positive OPSCC patients are typically younger, male (approximately 75%), and usually nonsmokers.[4,5] HPV-positive OPSCCs show distinctive biology and have better prognoses than HPV-negative OPSCCs, which has initiated the development of de-escalation treatment clinical trials that aim to promote positive results while reducing treatment-associated comorbidities.[6,7] Despite the better prognosis, about 20% of OPSCC patients will recur after 5 years of initial treatment, with an additional percentage of fatal cases.[6,8] Nevertheless, recent progress in OPSCC research has identified potential novel targets for precision medicine and early tumor detection.

Tumor microenvironment

The tumor microenvironment is the system around a tumor, including the blood vessels, immune cells, fibroblasts, signaling molecules, and the extracellular matrix. Most high-risk HPV infections are eliminated by the body's robust immune system, which recognizes viral antigens by T lymphocytes.[9] However, viral infections in HPV-positive OPSCCs persist by manipulating the immune system and surrounding micro-system. The tumor microenvironment (TME) includes various stromal cells such as rich lymphocytes and myeloid cells, fibroblasts, and endothelial cells, which interact with tumor cells.[9–12] The TME of OPSCCs is highly immunosuppressive by presenting immune checkpoint ligands, down-regulating human leukocyte antigen expression, inactivating the nuclear factor-kappa B pathway, causing cytotoxic T lymphocytes to malfunction, and activating immunosuppressive cell types, such as regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells (Fig. 2).[10–15] The immunomodulatory effect of HPV-related OPSCCs is also related to the HPV integration status. Compared to HPV-integrated OPSCCs, integration-negative tumors demonstrate significant elevation of genes expressed in natural killer cells, T-cells ($CD4^+$, regulatory, $CD3^+$, and $CD8^+$), and B cells. Integration-negative tumors are associated with better prognoses.[16] In HPV-positive OPSCCs, tumor-infiltrating lymphocytes (TILs) are reported to exert a protective function by way of an adaptive host immune response that targets viral antigens, leading to the identification of a specific subpopulation of lymphocytes that fights against HPV-associated cancer (HPV-16 E7 T cells).[17]

A series of efforts have been made to employ biomarkers from the TME, including TILs, to guide prognosis and precision immunotherapies for OPSCCs. Using the enrich-

Keywords: Tumor microenvironment; APOBEC3; DNA methylation; Novel therapeutics.

Abbreviations: APOBEC3, A3, apolipoprotein B mRNA editing enzyme catalytic subunit-like protein 3; HNC, head/neck cancer; HNSCCs, head and neck squamous cell carcinomas; HPV, human papillomavirus; OPSCC, oropharyngeal squamous cell carcinoma; TCGA, The Cancer Genome Atlas; TILs, tumor-infiltrating lymphocytes; TME, tumor microenvironment.

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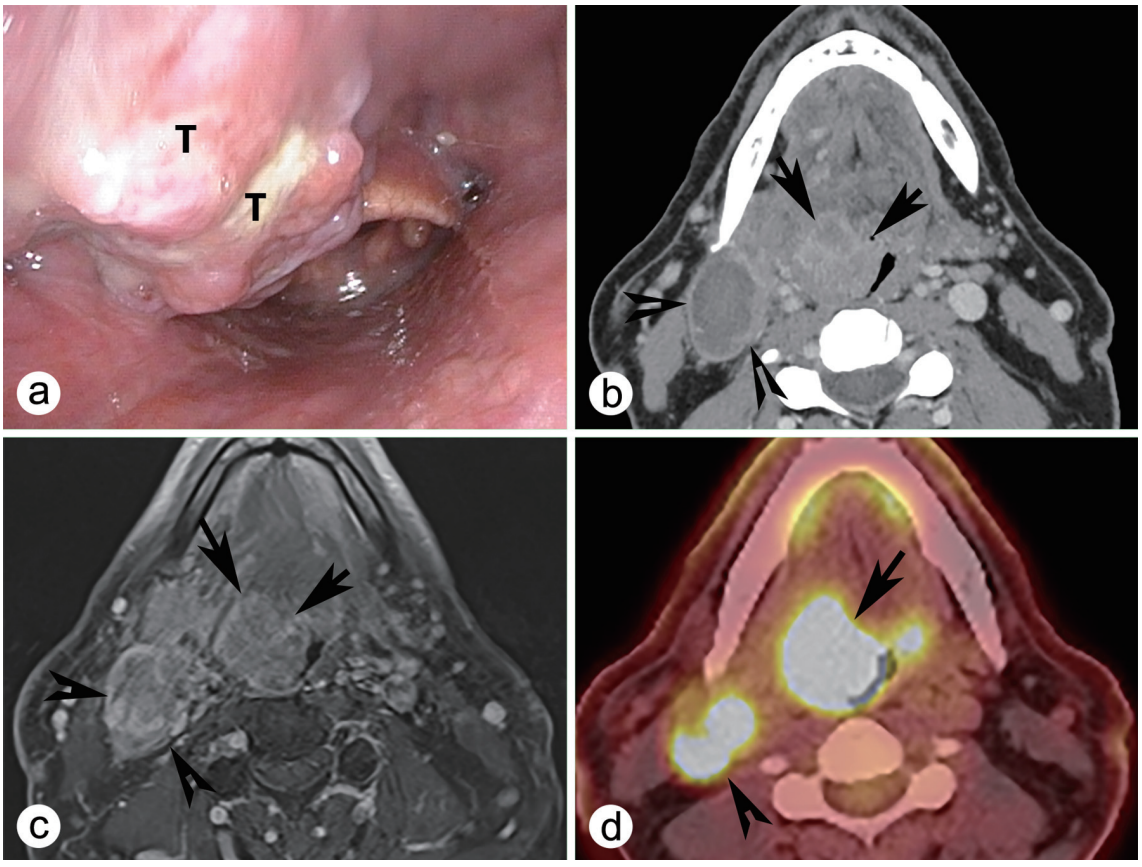

Fig. 1. Oropharyngeal squamous cell carcinoma. (a) A 66-year-old male with a 4-centimeter mass (T) involving the left base of the tongue and oropharynx with focal ulceration. (b–d) The computed tomography scan (b, axial), magnetic resonance image (c, T1, axial), and positron emission tomography/computed tomography scan (d, axial) showing a hyperintense base of the tongue lesion that extends into the floor of the mouth (arrows) and left neck level 2 lymph node metastasis (arrow heads).

ment scores of 33 immune cell types based on the gene expression data of OPSCC tissues and the surrounding benign tissues, Mito *et al*.[18] identified three types of immune signatures—cold, lymphocyte, and myeloid/dendritic cell. Most HPV-positive OPSCCs exhibit lymphocyte signatures, with several immune cell types, including $CD4^+$ T cells, $CD8^+$ T cells, B cells, and plasma cells, showing the highest scores. HPV-positive OPSCCs also demonstrate the longest overall survival.[18] Faraji *et al*.[19] also have revealed that an increased TIL density correlates with a low risk of recurrence in low-stage HPV-positive OPSCC. In contrast, decreased TIL infiltration, associated with cigarette exposure, has been linked to a higher stage at presentation and regional recurrence.[20] The spatial architecture patterns of TILs and the surrounding nucleated cells in hematoxylin and eosin-stained images of HPV-positive OPSCC patients can be characterized using P-TIL, an imaging biomarker, which can help differentiate stage I HPV-positive OPSCC patients into low- and high-risk subgroups and help patient triage for de-escalation.[21]

Aberrant DNA methylation and prognosis

HPV E6 and E7 regulate DNA methylation of the host genome in addition to inactivating p53 and retinoblastoma protein, respectively.[22–24] Earlier studies indicate that HPV-positive OPSCCs tend to contain higher amounts of aberrantly methylated DNA in the individual genes involved in cell-cycle regulation, cellular adhesion, cellular migration, apoptosis, and differentiation.[25,26] Recent comprehensive DNA methylation studies at the whole-genome level also have revealed an HPV-positive OPSCC subtype with DNA hypermethylation. Moreover, Ando *et al*.[27] have investigated the methylation profiles of HPV-positive head and neck squamous cell carcinomas (HNSCCs) and healthy mucosal samples and identified a group of 59 genes with a negative correlation between DNA methylation and RNA expression; furthermore, unsupervised hierarchical clustering analysis of the genes revealed a high-DNA-methylation phenotype in HPV-positive cases. Additional analysis of the 59 genes in The Cancer Genome Atlas (TCGA) OPSCC samples demonstrated the high-DNA-methylation phenotype. Likewise, Nakagawa *et al*.[28] found a high-DNA-methylation subtype in HPV-positive OPSCCs, which was positively correlated with an improved prognosis. After performing Infinium 450 k array analysis on 170 OPSCC samples, unsupervised hierarchical clustering with >1,000 probes showed that HPV-positive OPSCCs were stratified into two epigenotypes with distinct clinicopathological features. The HPV-positive, high-DNA-methylation phenotype had the best outcome among the HPV-positive OPSCC cases. It is known that HPV-positive HNSCCs have two different types of HPV infection patterns—HPV integration-positive and HPV integration-negative (episomal). In addition, Ren *et al*.[29] have shown that infection patterns correlate with HPV gene expression patterns. In HPV-positive HNSCC cases from the TCGA dataset, those with HPV integration-positive tumors showed a high expres-

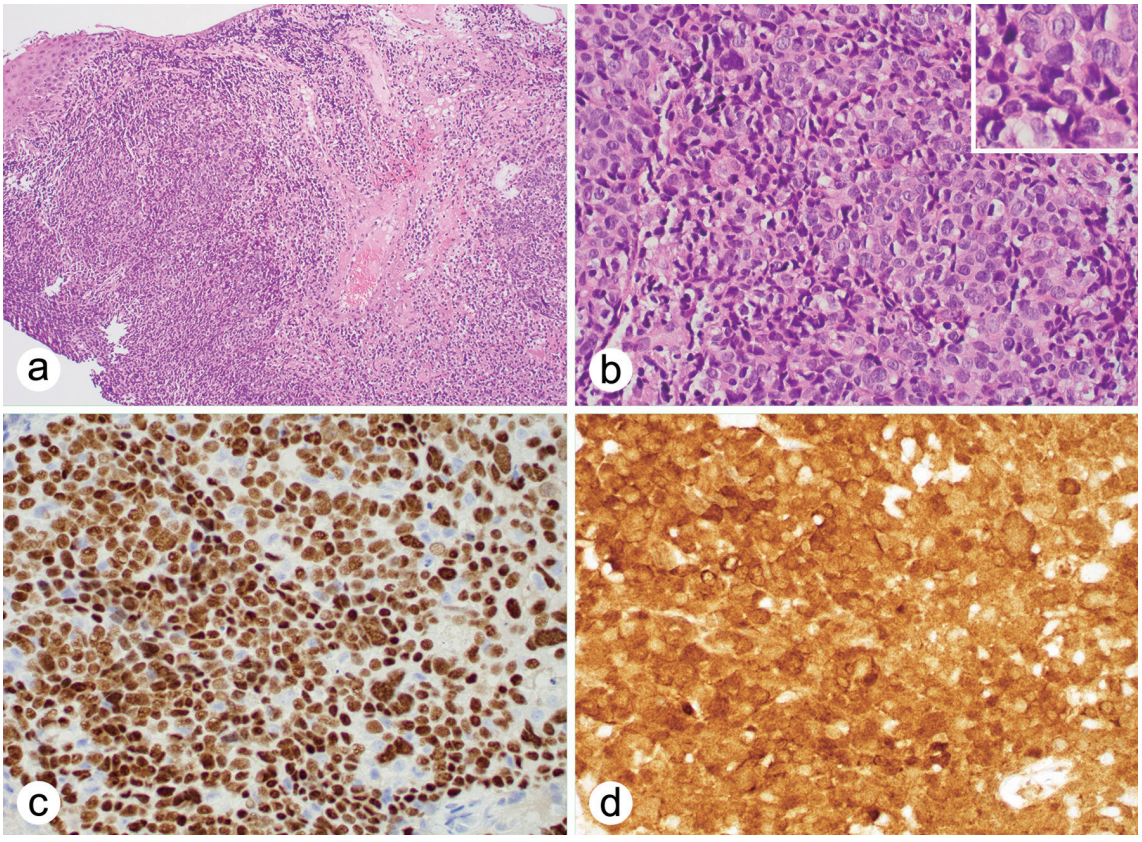

Fig. 2. Oropharyngeal squamous cell carcinoma. (a, b) Biopsy of the base of the tongue mass (stained with hematoxylin and eosin), shown in Figure 1, demonstrating the morphology of human papillomavirus-related squamous cell carcinoma with tumor-infiltrating lymphocytes (b, inset). (c, d) Immunohistochemistry showing that the tumor cells are positive for p40 (c) and p16 (d). (a, 100× magnification; b–d, 400× magnification).

sion of E6/E7 and a low expression of E2/E4/E5. In contrast, those with HPV integration-negative tumors showed an increased expression of E2/E4/E5 and a low expression of E6/E7. These findings were validated using an independent HPV-related OPSCC set and a TCGA cervical cancer cohort. When the HPV genome integrates into the human genome, E2 is usually disrupted, resulting in the upregulation of E6/E7.[30–32] Although further analysis is needed, the upregulated E6 and E7 genes may be associated with the different HNSCC subtype DNA methylation patterns.

Apolipoprotein B mRNA editing enzyme catalytic subunit-like protein 3

Whole-genome sequencing studies of various cancers over the last decade have led to an important conclusion: almost all HPV-positive head/neck cancer (HNC), in addition to many HPV-negative HNC and cancers from several other organ systems, have a high percentage of somatic mutations linked to members of the apolipoprotein B mRNA editing enzyme catalytic subunit-like protein 3 (APOBEC3, A3) family.[33–38] APOBEC3-induced mutations include cancer driver mutations like the activating mutations in PIK3CA.[39,40] APOBEC-mediated mutational signatures are found, with striking similarity, in viral and cancer genomes. The APOBEC3 mutation signature is defined by C-to-T and C-to-G changes in 5′-TCA and 5′-TCT trinucleotide motifs.[37,38] The human APOBEC3 family includes seven enzymes, A3A–D and A3F–H. In HPV-positive and HPV-negative HNCs, current evidence points to APOBEC3A (A3A) and APOBEC3B (A3B) as the most likely sources of the overall APOBEC mutation signature. As part of the innate immune response following high-risk HPV infections, both A3A and A3B are upregulated by HPV oncoproteins (E6 and E7) and by interferon,[40–45] potentially to promote viral genetic diversification, while collateral host genomic DNA damage contributes to carcinogenesis. Law *et al*.[46] have demonstrated that in a murine adenomatous polyposis coli multiple intestinal neoplasia model, transgenic overexpression of human A3A, but not A3G, resulted in an increased incidence of polyp formation and induced C-to-T mutations in APOBEC-signature trinucleotide motifs. All seven human A3 enzymes in the murine fumaryl-acetoacetate hydrolase model for hepatocellular carcinoma, including A3A, A3B, and A3H, were tested sequentially. However, only human A3A significantly increased the frequencies of hepatocellular tumors to above control levels. The most substantial evidence that APOBEC3 enzymes drive tumor evolution in humans is their positive associations with poor clinical outcomes[47,48] and significant increases in the proportion of APOBEC3 signature mutations from primary to metastatic disease.[49,50]

One remaining question is whether viral editing and host genome mutations are concurrently linked events mediated by the same A3. In one recent study, Faden *et al*.[51] quantified the AAPOBEC mutational burden and activity in both the host and virus by sequencing the host somatic exomes, transcriptomes, and HPV16 genomes from 79 HPV-positive human OPSCC specimens. They concluded that the primary mutational signature in somatic exomes is APOBEC. While there is a mean of five (range: 0–29) mutations per genome

in viral genomes, APOBEC mutations have a mean of one (range: 0–5). Compared to non-APOBEC mutations, viral APOBEC mutations are more often low-variant allele fraction mutations, indicating that APOBEC mutagenesis actively occurs in viral genomes during infection. HPV/APOBEC-induced mutation patterns in OPSCCs are similar to the mutation patterns observed in cervical tumors. Additionally, paired host and viral analyses reveal that APOBEC-enriched tumor samples have higher viral APOBEC mutation rates (p=0.028) and APOBEC-associated RNA editing (p=0.008), which further indicates that APOBEC mutagenesis in host and viral genomes is directly linked and likely occurs during infection. The above observation supports the general hypothesis[52] that HPV induces A3 activity, possibly to generate variation in viral progeny. However, albeit infrequently, mutations in cancer-causing genes such as *PIK3CA* can result from other viral activities such as the induction of replication stress, which can cause A3 activity against the host genome. Although cells with these mutations gain a selective survival advantage, they can be suppressed for years by the host immune system. Therefore, tumor development, even in the beginning stages of HPV infection, can result from A3 activity.

Novel therapeutic approaches for HPV-positive OPSCCs

By promoting *de novo* or potentiating pre-existing antitumor immune responses, immune therapy for HPV-positive OPSCC has been developed based on our expanding knowledge of tumor immunology and the TME. As mentioned earlier, HPV-positive OPSCC belongs to the most immune-rich category of tumors,[53] and it is associated with infiltration of type I macrophages, natural killer cells, $CD4^+$ $CD25^+$ Tregs, $CD8^+$ $PD\text{-}1^+$ T cells, and high expression of PD-1, CTLA-4, and TIM-3 on the T cells.[54] Not surprisingly, HPV-positive OPSCC has repeatedly shown favorable responses to immune checkpoint inhibitors in animal models and clinical trials. For example, one clinical trial (Keynote-012) that evaluated pembrolizumab (an anti-PD-1 antibody) in refractory/metastatic HNSCC patients showed that although the overall objective response rate was 18%, subgroup analyses illustrated that the response rate was 25% in HPV-positive patients versus 14% in HPV-negative HNSCC patients.[54–56] In another multi-institutional, international durvalumab trial with an anti-PD-L1 antibody, the trial NCT02207530 showed an objective response rate of 16.2%, which translates into 29.4% in HPV-positive patients and 10.8% in HPV-negative patients.[57] Although immunotherapies do not directly target driver oncogenic mutations, they do affect immune cells in tumor nests and can influence tumor responses to treatments. Various immune checkpoint inhibitors, including anti-PD1, anti-PDL1, and CTLA-4 antibodies, have been tested to treat HNSCC; however, thus far, only anti-PD-1/PD-L1 antibodies have been approved for clinical use.[58–60] The anti-PD-1 antibodies nivolumab and pembrolizumab were granted Federal Drug Administration approval in 2016 to treat patients with metastatic, platinum-refractory HNSCC, with supporting data from the CheckMate-141 and KEYNOTE-040 trials, respectively. In 2019, pembrolizumab was further approved as a first-line therapy for patients with PD-L1-positive metastatic or unresectable HNSCC, with supporting data from the KEYNOTE-048 trial.[61–63]

Therapeutic vaccines transfer antigens to antigen-presenting cells and trigger cytotoxic T-cell and/or helper T-cell responses to get rid of existing tumors. A variety of therapeutic vaccines have been developed, including live vector (bacterial or viral vector), peptide, DNA/RNA, and whole cell-based vaccines.[64] In recent years, a series of therapeutic vaccines have been evaluated in clinical trials to treat patients with HPV-positive OPSCC. In addition, many studies are now evaluating the therapeutic effect of vaccines in combination with an immune checkpoint inhibitor or other immunomodulatory agent.[60,65] Early results from a few trials show promising results. For example, an objective response rate of 36% and a median overall duration of 17.5 months were observed in 22 HPV-positive OPSCC patients in a trial combining nivolumab with an HPV-16 E6/E7 peptide vaccine, and these results were superior to those from trials evaluating nivolumab alone.[66] In addition, in the MEDI0457 trial, a DNA vaccine encoding the E6 and E7 antigens was administered with DNA encoding interleukin-12, which induced lasting HPV-specific immune activity in 18 of 21 patients with locally advanced p16-positive HNSCC.[67] Another ongoing clinical trial is testing an E7-targeting mRNA vaccine delivered in combination with an agonistic anti-CD40 antibody. Moreover, a novel E6/E7-targeting vaccine, with or without immune checkpoint inhibitors, is being tested. The results of these vaccine trials will be the next cornerstone of immunotherapies for HPV-positive OPSCC patients.

DNA methylation is widespread in OPSCCs and substantially impacts tumor prognosis, so methylation seems to be a natural therapeutic target for HPV-positive OPSCC treatment. Epigenetic therapies of 5-azacytidine and 5-aza-20-deoxycytidine, which are cytidine analogs incorporated into DNA, lead to covalent adduct formation and work as DNA methyltransferase inhibitors.[68] The Federal Drug Administration has approved these therapies to treat certain myelodysplastic syndromes and chronic myelomonocytic leukemia cases, but their efficacy for solid cancers is still under consideration.[69] One ongoing clinical trial of 5-azacytidine aims to treat HPV-positive and HPV-negative HNSCCs (NCT02178072).

One critical question before making A3 a therapeutic target for HPV-positive OPSCC patients is whether A3 activity against the host genome is occurring at diagnosis and during treatment. More and more evidence suggests that A3 activity continues to generate mutations during treatment.[70,71] For example, a recent study in which suppression of A3B expression by inducible RNA interference delayed the acquisition of tamoxifen resistance in an *in vivo* xenografted breast cancer model supports the concept that A3 activity contributes to the evolution of therapeutic resistance.[72] This observation suggests a therapeutic benefit of inhibiting A3B and other A3 enzymes as an adjuvant to chemotherapy, a notion actively pursued in academia and industry alike.[72,73]

Conclusions

Significant progress has been made over the last five years in understanding the pathogenesis of HPV-positive OPSCCs, especially in the TME, APOBEC3-induced somatic mutations, aberrant DNA methylation, as well as novel therapeutics. Biomarkers based on the in-depth understanding of the abovementioned pathological processes, such as the density of tumor-infiltrating lymphocytes and high-DNA-methylation profiles, serve as new and refined prognostic markers for OPSCC patient survival, thus guiding the treatment strategy. In addition, emerging therapies based on the latest discoveries, including immune checkpoint inhibitors, APOBEC3B inhibitors, and DNA methyltransferase inhibitors, will provide additional precision treatments for OPSCC patients.

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Conflict of interest

JL has been an editorial board member since January 1, 2022, and HW has been a Deputy Editor-in-Chief of *Journal of Clinical and Translational Pathology* since May 2021. The authors have no other conflicts of interest related to this publication.

Author contributions

Study design (HW, QS, JL), analysis and interpretation of data (JC, HW, YX, KP), manuscript writing (JC, QS, YX, JL, KP, HW), critical revision (JC, QS, YX, JL, KP, HW), critical funding (HW). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

This is a review article, no Institutional Review Board approval was required. However, the written informed consent was obtained from the patient for publication of the images.

References

[1] Brown LM, Check DP, Devesa SS. Oropharyngeal cancer incidence trends: diminishing racial disparities. Cancer Causes Control 2011;22(5):753–763. doi:10.1007/s10552-011-9748-1, PMID:21380619.
[2] van Monsjou HS, van Velthuysen ML, van den Brekel MW, Jordanova ES, Melief CJ, Balm AJ. Human papillomavirus status in young patients with head and neck squamous cell carcinoma. Int J Cancer 2012;130(8):1806–1812. doi:10.1002/ijc.26195, PMID:21607949.
[3] Welters MJP, Ma W, Santegoets SJAM, Goedemans R, Ehsan I, Jordanova ES, *et al*. Intratumoral HPV16-Specific T Cells Constitute a Type I-Oriented Tumor Microenvironment to Improve Survival in HPV16-Driven Oropharyngeal Cancer. Clin Cancer Res 2018;24(3):634–647. doi:10.1158/1078-0432.CCR-17-2140, PMID:29018052.
[4] Wang HF, Wang SS, Tang YJ, Chen Y, Zheng M, Tang YL, *et al*. The Double-Edged Sword-How Human Papillomaviruses Interact With Immunity in Head and Neck Cancer. Front Immunol 2019;10:653. doi:10.3389/fimmu.2019.00653, PMID:31001266.
[5] Syrjänen S, Syrjänen K. HPV in Head and Neck Carcinomas: Different HPV Profiles in Oropharyngeal Carcinomas - Why? Acta Cytol 2019;63(2):124–142. doi:10.1159/000495727, PMID:30861518.
[6] Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, NguyenTân PF, *et al*. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363(1):24–35. doi:10.1056/NEJMoa0912217, PMID:20530316.
[7] Posner MR, Lorch JH, Goloubeva O, Tan M, Schumaker LM, Sarlis NJ, *et al*. Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase III trial. Ann Oncol 2011;22(5):1071–1077. doi:10.1093/annonc/mdr006, PMID:21317223.
[8] Du E, Mazul AL, Farquhar D, Brennan P, Anantharaman D, Abedi-Ardekani B, *et al*. Long-term Survival in Head and Neck Cancer: Impact of Site, Stage, Smoking, and Human Papillomavirus Status. Laryngoscope 2019;129(11):2506–2513. doi:10.1002/lary.27807, PMID:30637762.
[9] Doorbar J, Jenkins D, Stoler MH, Bergeron C. Biology of the Human Papillomavirus Life Cycle: The Basis for Understanding the Pathology of Precancer and Cancer. In: Jenkins D, Xavier Bosch F (eds). Human Papillomavirus. Cambridge, MA, USA: Academic Press. 2020:67–83. doi:10.1016/B978-0-12-814457-2.00005-2.
[10] Pan C, Issaeva N, Yarbrough WG. HPV-driven oropharyngeal cancer: current knowledge of molecular biology and mechanisms of carcinogenesis. Cancers Head Neck 2018;3:12. doi:10.1186/s41199-018-0039-3, PMID:31093365.
[11] Outh-Gauer S, Morini A, Tartour E, Lépine C, Jung AC, Badoual C. The Microenvironment of Head and Neck Cancers: Papillomavirus Involvement and Potential Impact of Immunomodulatory Treatments. Head Neck Pathol 2020;14(2):330–340. doi:10.1007/s12105-020-01147-x, PMID:32124416.
[12] Chen SMY, Krinsky AL, Woolaver RA, Wang X, Chen Z, Wang JH. Tumor immune microenvironment in head and neck cancers. Mol Carcinog 2020;59(7):766–774. doi:10.1002/mc.23162, PMID:32017286.
[13] Wondergem NE, Nauta IH, Muijlwijk T, Leemans CR, van de Ven R. The Immune Microenvironment in Head and Neck Squamous Cell Carcinoma: on Subsets and Subsites. Curr Oncol Rep 2020;22(8):81. doi:10.1007/s11912-020-00938-3, PMID:32602047.
[14] Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. Front Immunol 2020;11:940. doi:10.3389/fimmu.2020.00940, PMID:32499786.
[15] Mandal R, Şenbabaoğlu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, *et al*. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight 2016;1(17):e89829. doi:10.1172/jci.insight.89829, PMID:27777979.
[16] Koneva LA, Zhang Y, Virani S, Hall PB, McHugh JB, Chepeha DB, *et al*. HPV Integration in HNSCC Correlates with Survival Outcomes, Immune Response Signatures, and Candidate Drivers. Mol Cancer Res 2018;16(1):90–102. doi:10.1158/1541-7786.MCR-17-0153, PMID:28928286.
[17] Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, *et al*. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. Br J Cancer 2014;110(2):489–500. doi:10.1038/bjc.2013.639, PMID:24169344.
[18] Mito I, Takahashi H, Kawabata-Iwakawa R, Ida S, Tada H, Chikamatsu K. Comprehensive analysis of immune cell enrichment in the tumor microenvironment of head and neck squamous cell carcinoma. Sci Rep 2021;11(1):16134. doi:10.1038/s41598-021-95718-9, PMID:34373557.
[19] Faraji F, Fung N, Zaidi M, Gourin CC, Eisele DW, Rooper LM, *et al*. Tumor-infiltrating lymphocyte quantification stratifies early-stage human papillomavirus oropharynx cancer prognosis. Laryngoscope 2020;130(4):930–938. doi:10.1002/lary.28044, PMID:31070246.
[20] Kemnade JO, Elhalawani H, Castro P, Yu J, Lai S, Ittmann M, *et al*. CD8 infiltration is associated with disease control and tobacco exposure in intermediate-risk oropharyngeal cancer. Sci Rep 2020;10(1):243. doi:10.1038/s41598-019-57111-5, PMID:31937831.
[21] Corredor G, Toro P, Koyuncu C, Lu C, Buzzy C, Bera K, *et al*. An Imaging Biomarker of Tumor-Infiltrating Lymphocytes to Risk-Stratify Patients with HPV-Associated Oropharyngeal Cancer. J Natl Cancer Inst 2021:djab215. doi:10.1093/jnci/djab215, PMID:34850048.
[22] Au Yeung CL, Tsang WP, Tsang TY, Co NN, Yau PL, Kwok TT. HPV-16 E6 upregulation of DNMT1 through repression of tumor suppressor p53. Oncol Rep 2010;24(6):1599–1604. doi:10.3892/or_00001023, PMID:21042757.
[23] Burgers WA, Blanchon L, Pradhan S, de Launoit Y, Kouzarides T, Fuks F. Viral oncoproteins target the DNA methyltransferases. Oncogene 2007;26(11):1650–1655. doi:10.1038/sj.onc.1209950, PMID:16983344.
[24] Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer 2014;14(6):395–405. doi:10.1038/nrc3728, PMID:24854082.
[25] Nakagawa T, Matsusaka K, Misawa K, Ota S, Takane K, Fukuyo M, *et al*. Frequent promoter hypermethylation associated with human papillomavirus infection in pharyngeal cancer. Cancer Lett 2017;407:21–31. doi:10.1016/j.canlet.2017.08.008, PMID:28823962.
[26] Lechner M, Fenton T, West J, Wilson G, Feber A, Henderson S, *et al*. Identification and functional validation of HPV-mediated hypermethylation in head and neck squamous cell carcinoma. Genome Med 2013;5(2):15. doi:10.1186/gm419, PMID:23419152.
[27] Ando M, Saito Y, Xu G, Bui NQ, Medetgul-Ernar K, Pu M, *et al*. Chromatin dysregulation and DNA methylation at transcription start sites associated with transcriptional repression in cancers. Nat Commun 2019;10(1):2188. doi:10.1038/s41467-019-09937-w, PMID:31097695.
[28] Nakagawa T, Matsusaka K, Misawa K, Ota S, Fukuyo M, Rahmutulla B, *et al*. Stratification of HPV-associated and HPV-negative oropharyngeal squamous cell carcinomas based on DNA methylation epigenotypes. Int J Cancer 2020;146(9):2460–2474. doi:10.1002/ijc.32890, PMID:31997344.
[29] Ren S, Gaykalova DA, Guo T, Favorov AV, Fertig EJ, Tamayo P, *et al*. HPV E2, E4, E5 drive alternative carcinogenic pathways in HPV positive cancers. Oncogene 2020;39(40):6327–6339. doi:10.1038/s41388-020-01431-8, PMID:32848210.
[30] Romanczuk H, Howley PM. Disruption of either the E1 or the E2 regulatory gene of human papillomavirus type 16 increases viral immortalization capacity. Proc Natl Acad Sci USA 1992;89(7):3159–3163. doi:10.1073/pnas.89.7.3159, PMID:1313584.
[31] Vernon SD, Unger ER, Miller DL, Lee DR, Reeves WC. Association of human papillomavirus type 16 integration in the E2 gene with poor disease-free survival from cervical cancer. Int J Cancer 1997;74(1):50–56. doi:10.1002/(sici)1097-0215(19970220)74:1<50::aid-ijc9>3.0.co;2-#, PMID:9036869.
[32] Gray E, Pett MR, Ward D, Winder DM, Stanley MA, Roberts I, *et al*. In vitro progression of human papillomavirus 16 episome-associated cervical neoplasia displays fundamental similarities to integrant-associated carcinogenesis. Cancer Res 2010;70(10):4081–4091. doi:10.1158/0008-5472.CAN-09-3335, PMID:20442284.
[33] The Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517(7536):576–582. doi:10.1038/nature14129, PMID:25631445.
[34] Cannataro VL, Gaffney SG, Sasaki T, Issaeva N, Grewal NKS, Grandis JR, *et al*. APOBEC-induced mutations and their cancer effect size in head and neck squamous cell carcinoma. Oncogene 2019;38(18):3475–3487. doi:10.1038/s41388-018-0657-6, PMID:30647454.
[35] Faden DL, Thomas S, Cantalupo PG, Agrawal N, Myers J, DeRisi J. Multi-modality analysis supports APOBEC as a major source of mutations in head and neck squamous cell carcinoma. Oral Oncol 2017;74:8–14. doi:10.1016/j.oraloncology.2017.09.002, PMID:29103756.
[36] Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, *et*

al. APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 2013;494(7437):366–370. doi:10.1038/nature11881, PMID:23389445.
[37] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, *et al*. Signatures of mutational processes in human cancer. Nature 2013;500(7463):415–421. doi:10.1038/nature12477, PMID:23945592.
[38] Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, *et al*. The repertoire of mutational signatures in human cancer. Nature 2020; 578(7793):94–101. doi:10.1038/s41586-020-1943-3, PMID:32025018.
[39] Henderson S, Chakravarthy A, Su X, Boshoff C, Fenton TR. APOBEC-mediated cytosine deamination links PIK3CA helical domain mutations to human papillomavirus-driven tumor development. Cell Rep 2014;7(6):1833–1841. doi:10.1016/j.celrep.2014.05.012, PMID:24910434.
[40] Faden DL, Ding F, Lin Y, Zhai S, Kuo F, Chan TA, *et al*. APOBEC mutagenesis is tightly linked to the immune landscape and immunotherapy biomarkers in head and neck squamous cell carcinoma. Oral Oncol 2019;96:140–147. doi:10.1016/j.oraloncology.2019.07.020, PMID:31422205.
[41] Vieira VC, Leonard B, White EA, Starrett GJ, Temiz NA, Lorenz LD, *et al*. Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B. mBio 2014;5(6):e02234–14. doi:10.1128/mBio.02234-14, PMID:25538195.
[42] Mori S, Takeuchi T, Ishii Y, Kukimoto I. Identification of APOBEC3B promoter elements responsible for activation by human papillomavirus type 16 E6. Biochem Biophys Res Commun 2015;460(3):555–560. doi:10.1016/j.bbrc.2015.03.068, PMID:25800874.
[43] Mori S, Takeuchi T, Ishii Y, Yugawa T, Kiyono T, Nishina H, *et al*. Human Papillomavirus 16 E6 Upregulates APOBEC3B via the TEAD Transcription Factor. J Virol 2017;91(6):e02413–16. doi:10.1128/JVI.02413-16, PMID:28077648.
[44] Westrich JA, Warren CJ, Klausner MJ, Guo K, Liu CW, Santiago ML, *et al*. Human Papillomavirus 16 E7 Stabilizes APOBEC3A Protein by Inhibiting Cullin 2-Dependent Protein Degradation. J Virol 2018;92(7):e01318–17. doi:10.1128/JVI.01318-17, PMID:29367246.
[45] Warren CJ, Xu T, Guo K, Griffin LM, Westrich JA, Lee D, *et al*. APOBEC3A functions as a restriction factor of human papillomavirus. J Virol 2015;89(1):688–702. doi:10.1128/JVI.02383-14, PMID:25355878.
[46] Law EK, Levin-Klein R, Jarvis MC, Kim H, Argyris PP, Carpenter MA, *et al*. APOBEC3A catalyzes mutation and drives carcinogenesis in vivo. J Exp Med 2020;217(12):e20200261. doi:10.1084/jem.20200261, PMID:32870257.
[47] Argyris PP, Wilkinson PE, Jarvis MC, Magliocca KR, Patel MR, Vogel RI, *et al*. Endogenous APOBEC3B overexpression characterizes HPV-positive and HPV-negative oral epithelial dysplasias and head and neck cancers. Mod Pathol 2021;34(2):280–290. doi:10.1038/s41379-020-0617-x, PMID:32632179.
[48] Meng F, Zhang L, Ren Y, Ma Q. The genomic alterations of lung adenocarcinoma and lung squamous cell carcinoma can explain the differences of their overall survival rates. J Cell Physiol 2019;234(7):10918–10925. doi:10.1002/jcp.27917, PMID:30549039.
[49] Bertucci F, Ng CKY, Patsouris A, Droin N, Piscuoglio S, Carbuccia N, *et al*. Genomic characterization of metastatic breast cancers. Nature 2019;569(7757):560–564. doi:10.1038/s41586-019-1056-z, PMID:31118521.
[50] Angus L, Smid M, Wilting SM, van Riet J, Van Hoeck A, Nguyen L, *et al*. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. Nat Genet 2019;51(10):1450–1458. doi:10.1038/s41588-019-0507-7, PMID:31570896.
[51] Faden DL, Kuhs KAL, Lin M, Langenbucher A, Pinheiro M, Yeager M, *et al*. APOBEC Mutagenesis Is Concordant between Tumor and Viral Genomes in HPV-Positive Head and Neck Squamous Cell Carcinoma. Viruses 2021;13(8):1666. doi:10.3390/v13081666, PMID:34452530.
[52] Smith NJ, Fenton TR. The APOBEC3 genes and their role in cancer: insights from human papillomavirus. J Mol Endocrinol 2019;62(4):R269–R287. doi:10.1530/JME-19-0011, PMID:30870810.
[53] Kim MH, Kim JH, Lee JM, Choi JW, Jung D, Cho H, *et al*. Molecular subtypes of oropharyngeal cancer show distinct immune microenvironment related with immune checkpoint blockade response. Br J Cancer 2020;122(11):1649–1660. doi:10.1038/s41416-020-0796-8, PMID:32235905.
[54] Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK, *et al*. Heterogeneity of the Head and Neck Squamous Cell Carcinoma Immune Landscape and Its Impact on Immunotherapy. Front Cell Dev Biol 2019;7:52. doi:10.3389/fcell.2019.00052, PMID:31024913.
[55] Gao P, Lazare C, Cao C, Meng Y, Wu P, Zhi W, *et al*. Immune checkpoint inhibitors in the treatment of virus-associated cancers. J Hematol Oncol 2019;12(1):58. doi:10.1186/s13045-019-0743-4, PMID:31182108.
[56] Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, *et al*. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol 2016;17(7):956–965. doi:10.1016/S1470-2045(16)30066-3, PMID:27247226.
[57] Zandberg DP, Algazi AP, Jimeno A, Good JS, Fayette J, Bouganim N, *et al*. Durvalumab for recurrent or metastatic head and neck squamous cell carcinoma: Results from a single-arm, phase II study in patients with ≥25% tumour cell PD-L1 expression who have progressed on platinum-based chemotherapy. Eur J Cancer 2019;107:142–152. doi:10.1016/j.ejca.2018.11.015, PMID:30576970.
[58] Frazer IH, Chandra J. Immunotherapy for HPV associated cancer. Papillomavirus Res 2019;8:100176. doi:10.1016/j.pvr.2019.100176, PMID:31310819.
[59] Barra F, Della Corte L, Noberasco G, Foreste V, Riemma G, Di Filippo C, *et al*. Advances in therapeutic vaccines for treating human papillomavirus-related cervical intraepithelial neoplasia. J Obstet Gynaecol Res 2020;46(7):989–1006. doi:10.1111/jog.14276, PMID:32390320.
[60] Smalley Rumfield C, Pellom ST, Morillon Ii YM, Schlom J, Jochems C. Immunomodulation to enhance the efficacy of an HPV therapeutic vaccine. J Immunother Cancer 2020;8(1):e000612. doi:10.1136/jitc-2020-000612, PMID:32554612.
[61] Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, *et al*. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med 2016;375(19):1856–1867. doi:10.1056/NEJMoa1602252, PMID:27718784.
[62] Cohen EEW, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, *et al*. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet 2019;393(10167):156–167. doi:10.1016/S0140-6736(18)31999-8, PMID:30509740.
[63] Burtness B, Harrington , Greil R, Soulières D, Tahara M, de Castro G Jr, *et al*. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet 2019;394(10212):1915–1928. doi:10.1016/S0140-6736(19)32591-7, PMID:31679945.
[64] Yang A, Farmer E, Lin J, Wu TC, Hung CF. The current state of therapeutic and T cell-based vaccines against human papillomaviruses. Virus Res 2017;231:148–165. doi:10.1016/j.virusres.2016.12.002, PMID:27932207.
[65] von Witzleben A, Wang C, Laban S, Savelyeva N, Ottensmeier CH. HNSCC: Tumour Antigens and Their Targeting by Immunotherapy. Cells 2020;9(9):E2103. doi:10.3390/cells9092103, PMID:32942747.
[66] Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, *et al*. Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A Phase 2 Clinical Trial. JAMA Oncol 2019;5(1):67–73. doi:10.1001/jamaoncol.2018.4051, PMID:30267032.
[67] Aggarwal C, Cohen RB, Morrow MP, Kraynyak KA, Sylvester AJ, Knoblock DM, *et al*. Immunotherapy Targeting HPV16/18 Generates Potent Immune Responses in HPV-Associated Head and Neck Cancer. Clin Cancer Res 2019;25(1):110–124. doi:10.1158/1078-0432.CCR-18-1763, PMID:30242022.
[68] Nervi C, De Marinis E, Codacci-Pisanelli G. Epigenetic treatment of solid tumours: a review of clinical trials. Clin Epigenetics 2015;7:127. doi:10.1186/s13148-015-0157-2, PMID:26692909.
[69] Linnekamp JF, Butter R, Spijker R, Medema JP, van Laarhoven HWM. Clinical and biological effects of demethylating agents on solid tumours - A systematic review. Cancer Treat Rev 2017;54:10–23. doi:10.1016/j.ctrv.2017.01.004, PMID:28189913.
[70] Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, *et al*. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. Nat Med 2016;22(3):262–269. doi:10.1038/nm.4040, PMID:26828195.
[71] Faltas BM, Prandi D, Tagawa ST, Molina AM, Nanus DM, Sternberg C, *et al*. Clonal evolution of chemotherapy-resistant urothelial carcinoma. Nat Genet 2016;48(12):1490–1499. doi:10.1038/ng.3692, PMID:27749842.
[72] Law EK, Sieuwerts AM, LaPara K, Leonard B, Starrett GJ, Molan AM, *et al*. The DNA cytosine deaminase APOBEC3B promotes tamoxifen resistance in ER-positive breast cancer. Sci Adv 2016;2(10):e1601737. doi:10.1126/sciadv.1601737, PMID:27730215.
[73] Olson ME, Harris RS, Harki DA. APOBEC Enzymes as Targets for Virus and Cancer Therapy. Cell Chem Biol 2018;25(1):36–49. doi:10.1016/j.chembiol.2017.10.007, PMID:29153851.