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Original Article

APOBEC3B/ASF1B–TGF-β signaling axis promotes epithelial–mesenchymal transition in HPV-positive oropharyngeal cancer

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Oropharyngeal cancer;
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Abstract *Background/purpose:* Human papillomavirus (HPV)-positive oropharyngeal cancer (OPC) generally shows better outcomes, yet a subset of patients develops aggressive lymphatic metastasis. The molecular determinants of this divergence remain unclear. This study explored the apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B)/anti-silencing function 1B histone chaperone (ASF1B) axis as a potential mediator linking human papillomavirus (HPV) infection, DNA damage, and transforming growth factor beta (TGF-β) signaling in OPC.
Materials and methods: Transcriptomic and clinical datasets from The Cancer Genome Atlas (TCGA) were analyzed to examine correlations among APOBEC3B/ASF1B expression, HPV status, and TGF-β signaling activity. Functional assays using HPV-positive and HPV-negative cell models assessed APOBEC3B/ASF1B expression, replication protein A2 (RPA2) activation, and epithelial–mesenchymal transition (EMT) markers through quantitative polymerase chain reaction (qPCR), western blotting, and immunofluorescence.
Results: HPV infection markedly enhanced APOBEC3B and ASF1B expression, accompanied by RPA2 upregulation and EMT features. Silencing APOBEC3B reduced RPA2 and ASF1B levels and suppressed TGF-β signaling.
Conclusion: These findings identify APOBEC3B/ASF1B as a central pathway through which HPV

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infection activates TGF-β signaling and promotes EMT, offering potential biomarkers and therapeutic targets for high-risk HPV-positive OPC.

Introduction

Prolonged exposure to carcinogens remains a principal cause of head and neck cancers worldwide. Tobacco use and heavy alcohol consumption particularly of distilled spirits are recognized as major contributors.[1] In parts of Asia, including Taiwan and southern China, habitual betel nut chewing adds a further carcinogenic burden, especially to the upper aerodigestive tract encompassing the oral cavity, pharynx, larynx, and esophagus.[2] In Taiwan, the incidence of these malignancies peaked around 2014 and has since stabilized, reflecting the positive impact of public health measures such as tobacco and betel nut control.[3] As these traditional risk factors gradually decline, the epidemiologic landscape is shifting toward an increased prevalence of human papillomavirus (HPV)—associated oropharyngeal cancers, particularly those arising in the tonsils and base of the tongue.[4] Among the emerging molecular players in HPV-related tumorigenesis, the apolipoprotein B mRNA editing catalytic polypeptide-like 3 (APOBEC3) family has garnered growing attention.[5] Originally characterized for its antiviral role against HIV, the APOBEC3 enzyme catalyzes cytosine-to-uracil deamination in single-stranded DNA, thereby introducing mutations that restrict viral replication.[6] However, aberrant apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B) activity in host cells can induce genome instability, contributing to somatic mutations found in a wide spectrum of human cancers including those of the breast, cervix, bladder, lung, ovary, and head and neck.[7] These characteristic APOBEC-related mutational signatures highlight its dual role as both an antiviral defense and a driver of carcinogenesis.[8]

Recent studies suggest that APOBEC3B expression is frequently upregulated in HPV-positive cancers and may interact with the anti-silencing function 1B histone chaperone (ASF1B) to modulate DNA replication stress and repair pathways.[9] We hypothesize that this APOBEC3B/ASF1B signaling axis contributes to the development of radioresistance and metastatic progression in oropharyngeal cancer (OPC). Elevated APOBEC3B levels following HPV infection appear to activate TGF-β signaling, leading to the induction of RPA2 and ASF1B, which promote cell proliferation and epithelial—mesenchymal transition (EMT). Conversely, silencing APOBEC3B reduces replication protein A2 (RPA2) and ASF1B expression and attenuates these malignant phenotypes. Based on these findings, we propose that targeting APOBEC3B may suppress the invasiveness and migratory capacity of radioresistant OPC cells. Moreover, combining APOBEC3B inhibition with cisplatin therapy could enhance apoptotic responses, offering a potential strategy to overcome treatment resistance and improve therapeutic efficacy in high-risk HPV-positive OPC.

Materials and methods

Cell lines and establishment of irradiation-resistant derivatives

Human oropharyngeal squamous cell carcinoma cell lines SCC-4 (HPV-negative) and SCC-154 (HPV-positive) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10 % fetal bovine serum (FBS; HyClone, Logan, UT, USA) and 1 % penicillin—streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C in a humidified 5 % CO_2 atmosphere. Mycoplasma contamination was routinely excluded using the MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland). To generate irradiation-resistant (IR-R) sub-lines, parental SCC-4 and SCC-154 cells were subjected to fractionated X-ray irradiation using an X-RAD 320 irradiator (Precision X-Ray, North Branford, CT, USA) at a dose of 2 Gy every 48 h, repeated for 30 cycles (totaling 60 Gy).After completion of 30 cycles, surviving cells were expanded and designated IR—SCC—4 and IR—SCC—154, respectively. Confirmation of radioresistance was performed by exposing parental and IR-R cells to a single dose of 0.5, 1, or 2 Gy and assessing viability at 72 h post-irradiation using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA).

Migration and invasion assays

Cell migration was assessed using the scratch-wound assay. Confluent monolayers were scratched with a sterile 200 μL pipette tip, washed with PBS, and incubated in serum-free medium for 24—48 h. Images were captured using an inverted microscope, and migration distance was quantified at five random fields using Image J software (National Institutes of Health, Bethesda, MD, USA). For invasion assays, 3×10^5 cells were seeded in Matrigel-coated Transwell chambers (8 μm pore size; BD Biosciences, San Jose, CA, USA) with serum-free medium in the upper chamber and 10 % FBS in the lower chamber. After 24 h, invaded cells were fixed with 4 % paraformaldehyde, stained with 0.1 % crystal violet, and counted in five random fields under a microscope.

Tumor spheroid formation assay

To assess the effect of APOBEC3B knockdown on tumor-initiating capacity, HPV-positive (SCC-154) and HPV-negative (SCC-4) oral squamous cell carcinoma (OSCC) cells were cultured under anchorage-independent conditions to induce spheroid formation. Briefly, cells were suspended in serum-free DMEM/F-12 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with N2 supplement (Invitrogen, Carlsbad, CA, USA), 20 ng/mL epidermal growth factor (EGF) and 20 ng/mL basic fibroblast growth factor (bFGF) (PeproTech, Rocky Hill, NJ, USA). Approximately 2×10^4 cells were plated in ultra-low-attachment 6-well plates (Corning, Corning, NY, USA) and maintained for 14 days at 37 °C in a humidified 5 % CO_2 incubator.Fresh growth factors were replenished every 3 days. Tumor spheroids were visualized using an Olympus CKX53 inverted microscope (Olympus, Tokyo, Japan), and spheroids with diameters $\geq$75 μm were counted in five randomly selected fields per well using ImageJ (NIH, Bethesda, MD, USA). For APOBEC3B knockdown experiments, stable shRNA-expressing SCC-154 cells were generated as described above. The number and average size of spheroids formed by shAPOBEC3B cells were compared with non-targeting shRNA controls to evaluate changes in self-renewal and tumorigenic potential.Each experiment was performed in independent triplicates, and results are presented as mean ± SD.

RNA isolation and quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa Bio, Taipei, Taiwan). Quantitative real-time PCR (qPCR) was performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Relative mRNA expression levels of APOBEC3B, ASF1B, RPA2, and EMT markers were normalized to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) using the $2^{-}\Delta\Delta Ct$ method. The PCR primer design is shown in Supplementary Table 1.

shRNA-mediated gene silencing

Lentiviral particles carrying short hairpin RNA (shRNA) targeting APOBEC3B or non-targeting control shRNA were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Transductions were performed in BSL-2 facilities at the Translational Medicine Laboratory, Shuang Ho Hospital, Taipei Medical University (New Taipei city, Taiwan). Stable clones were selected with 2 μg/mL puromycin (Sigma—Aldrich, St. Louis, MO, USA). Gene knockdown efficiency was confirmed by qPCR.

Bioinformatics analysis

Transcriptomic data and clinical information for head and neck squamous cell carcinoma (HNSCC) were obtained from The Cancer Genome Atlas (TCGA) database and the GSE65858 dataset from the Gene Expression Omnibus (GEO).The GSE65858 cohort comprises comprehensive transcriptomic profiles and clinical annotations from 270 HNSCC patients, including HPV-positive and HPV-negative oropharyngeal cancer (OPSCC) cases, making it a well-established validation dataset for HPV-related transcriptional analyses. Expression correlations among APOBEC3B, ASF1B, and TGF-β signaling—related genes were analyzed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria).Differential expression and survival analyses were performed to validate findings across both TCGA and GEO cohorts.Survival outcomes were assessed using the Kaplan—Meier method, and pathway enrichment was evaluated via Gene Set Enrichment Analysis (GSEA; Broad Institute, Cambridge, MA, USA).

Statistical analysis

All experiments were performed independently at least three times. Data are presented as the mean ± standard deviation (SD). Statistical analyses between two groups were conducted using a two-tailed Student's t-test with GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). A $P < 0.05$ was considered statistically significant.

Results

High APOBEC3B expression predicts poor survival in HPV-positive oropharyngeal cancer

Analysis of The Cancer Genome Atlas (TCGA—HNSC) dataset revealed that APOBEC3B expression was significantly elevated in head and neck squamous cell carcinoma (HNSCC) compared with adjacent normal mucosa (Fig. 1A, $P < 0.001$, unpaired t-test). Data were obtained and visualized using UALCAN (http://ualcan.path.uab.edu) and GEPIA2 (http://gepia2.cancer-pku.cn), confirming the consistent overexpression of APOBEC3B across multiple patient cohorts. Kaplan—Meier survival analysis using KMplotter (https://kmplot.com) demonstrated that patients with high APOBEC3B levels exhibited significantly poorer overall survival compared with those with low expression (hazard ratio = 1.87, 95 % CI = 1.15—3.04, log-rank $P = 0.012$). When stratified by HPV status, this effect was most pronounced in HPV-positive patients, where elevated APOBEC3B expression strongly correlated with shorter survival (Fig. 1B and C). These results suggest that APOBEC3B upregulation is associated with an unfavorable clinical course specifically in HPV-driven oropharyngeal cancers. To identify molecular partners linked to APOBEC3B activity, we performed a co-expression analysis using the LinkedOmics database (http://www.linkedomics.org). Among the top correlated genes, ASF1B, Minichromosome Maintenance Complex Component 5 (MCM5), and Establishment of Sister Chromatid Cohesion N-Acetyltransferase 2 (ESCO2) key regulators of DNA replication and chromatin assembly—showed strong positive correlation with APOBEC3B (Pearson $r > 0.6$, $P < 0.001$). Illustrated in Fig. 1D, unveiled a striking trend: HPV-positive patients exhibited a significantly lower overall survival rate when compared to their HPV-negative

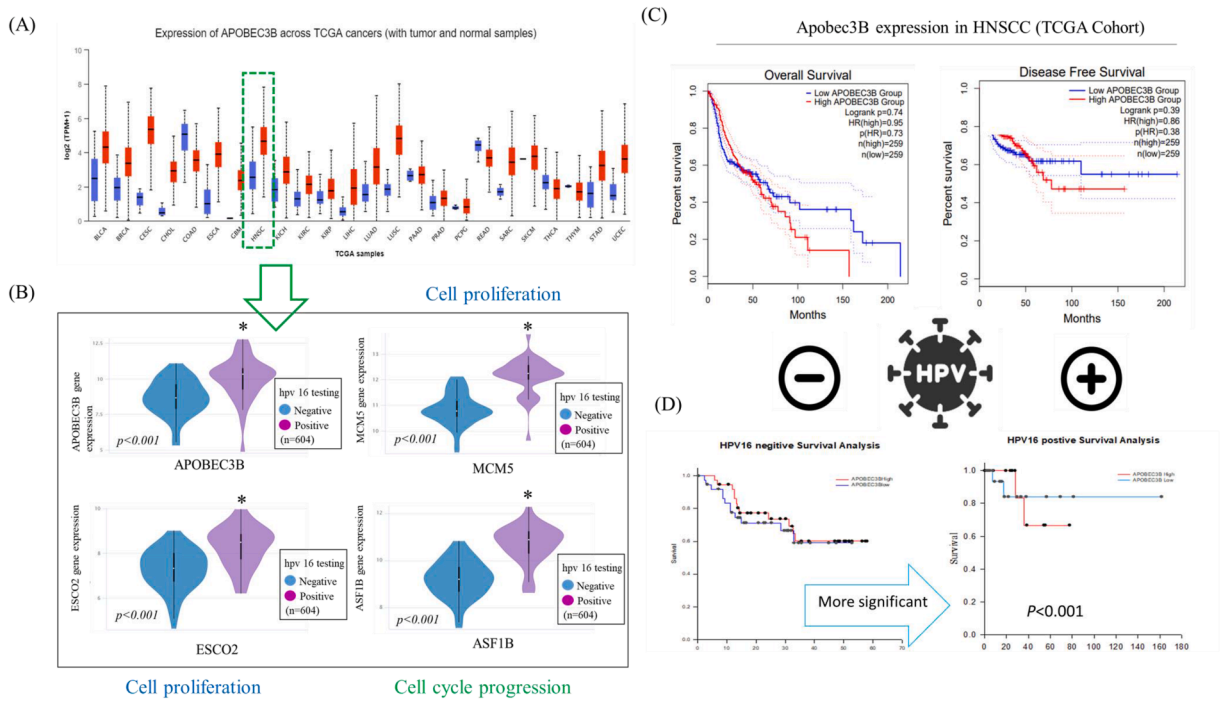
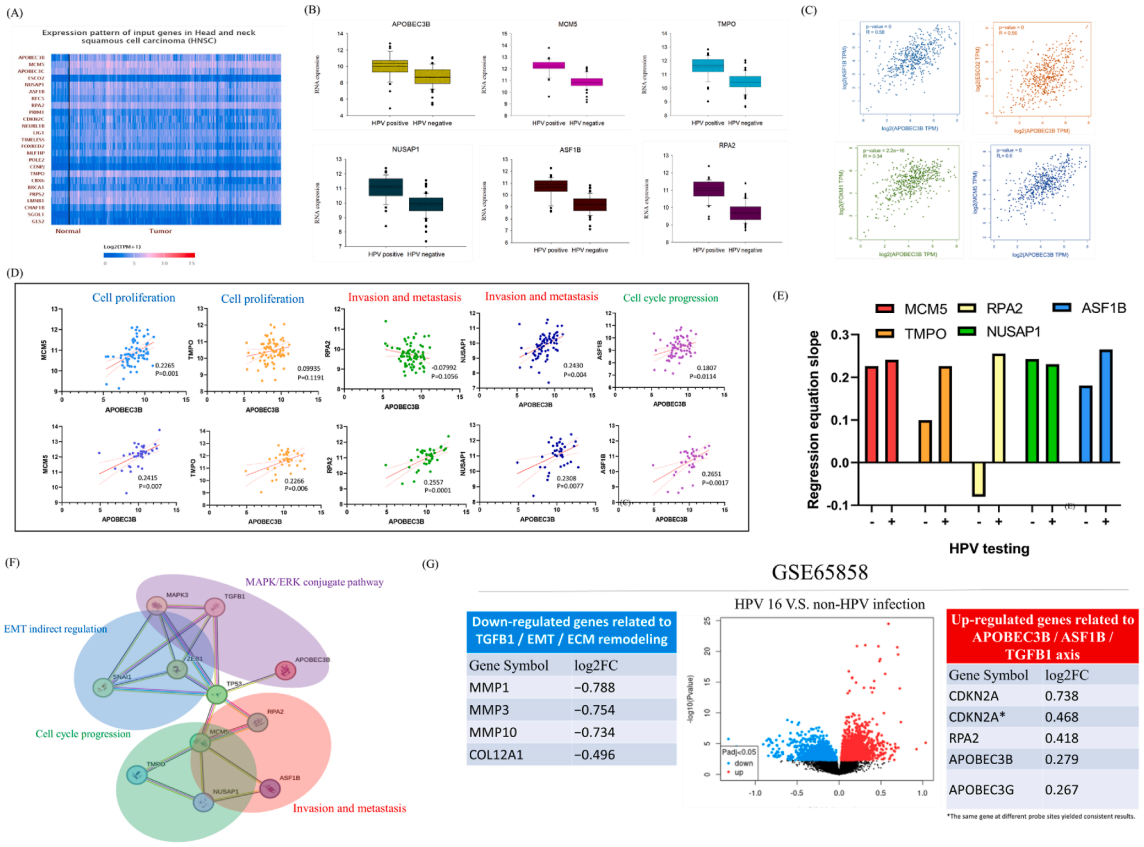


Figure 1 APOBEC3B overexpression and its clinical impact in HPV-positive head and neck cancer in the TCGA-HNSC cohort. (A) Differential expression analysis of APOBEC3B across multiple human cancers using the TCGA database shows marked upregulation in head and neck squamous cell carcinoma compared with normal tissue. (B) Expression profiles of MCM5, ESCO2, and ASF1B—genes involved in DNA replication and cell-cycle regulation—demonstrate significant co-upregulation in HPV-positive HNSCC. (C–D) Kaplan–Meier survival analysis indicates that high APOBEC3B expression correlates with poorer overall survival, an effect particularly evident in HPV-positive patients. Data were obtained from UALCAN, GEPIA2, and KMplotter using TCGA-HNSC RNA-seq datasets. Statistical significance was determined by unpaired t-test and log-rank test ($P < 0.05$). **Abbreviations:** HNSCC, head and neck squamous cell carcinoma; TCGA, The Cancer Genome Atlas; HPV, human papillomavirus; EMT, epithelial–mesenchymal transition.

counterparts. This trend not only corroborates our initial clinical observations but also underscores a potential link between elevated APOBEC3B expression and a poorer prognosis in HPV-positive head and neck cancer patients. The findings from our research underscore the importance of considering genetic and molecular profiles in the management of head and neck cancer. These analyses highlight APOBEC3B as a potential oncogenic driver in HPV-positive oropharyngeal cancer. Its coordinated upregulation with ASF1B, MCM5, and ESCO2 suggests an interplay between cytidine deamination, DNA replication, and repair processes that may underlie tumor aggressiveness and treatment resistance. The strong association between elevated APOBEC3B expression and poor overall survival emphasizes its potential value as a prognostic biomarker and therapeutic target in HPV-driven head and neck malignancies.

Correlation analysis of APOBEC3B and related genes in HPV-positive tissue with cancer cell EMT function

We analyzed the expression heat map of the APOBEC3B axis in the TCGA-HNSC database (Fig. 2A) and classified several genes associated with HPV infection status and tumor cell behavior, including APOBEC3B, MCM5, Thymopoietin (TMPO), Nucleolar and Spindle Associated Protein 1 (NUSAP1), ASF1B, and RPA2. The results showed that these genes were markedly upregulated in HPV-positive oral squamous cell carcinoma tissues compared with HPV-negative cases (Fig. 2B). Correlation analysis using Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn) demonstrated strong positive associations between APOBEC3B and its related genes, notably ASF1B, TMPO, and RPA2, which are implicated in DNA replication, cell proliferation, and migration (Fig. 2C). Further comparison of HPV-positive and HPV-negative subgroups revealed that TMPO (cell proliferation) and RPA2 (cell migration and invasion) showed significantly stronger correlations with APOBEC3B expression in HPV-positive tumors (Fig. 2D and E), suggesting viral modulation of this regulatory network. A gene interaction network generated using String (https://string-db.org/)visualized the tandem and signaling relationships among these genes (Fig. 2F). Furthermore, analysis of the GSE65858 cohort revealed co-expression of APOBEC3B and ASF1B in proliferating basal epithelial and mesenchymal-like cell subsets, highlighting their potential role in epithelial–mesenchymal transition (EMT) and metastasis.The downregulated cluster comprising MMPs, COL12A1, and TGFBI indicates suppression of the TGF-β–ECM remodeling/EMT pathway, potentially reflecting microenvironmental adaptation following



Down-regulated genes related to TGFB1 / EMT / ECM remodeling	
Gene Symbol	log2FC
MMP1	−0.788
MMP3	−0.754
MMP10	−0.734
COL12A1	−0.496

Up-regulated genes related to APOBEC3B / ASF1B / TGFB1 axis	
Gene Symbol	log2FC
CDKN2A	0.738
CDKN2A*	0.468
RPA2	0.418
APOBEC3B	0.279
APOBEC3G	0.267

*The same gene at different probe sites yielded consistent results.

Figure 2 Correlation and network analysis of APOBEC3B-associated genes in HPV-positive head and neck cancer and their link to epithelial—mesenchymal transition (EMT). (A) Heatmap showing expression profiles of the APOBEC3B axis and related genes in the TCGA-HNSC cohort. (B) Comparison of APOBEC3B, MCM5, TMPO, NUSAP1, ASF1B, and RPA2 expression between HPV-positive and HPV-negative oral squamous cell carcinoma tissues. (C—D) Correlation analysis performed using GEPIA2 (http://gepia2.cancer-pku.cn) demonstrates strong positive associations between APOBEC3B and its co-expressed genes.(E) Regression slope analysis comparing correlation strength across HPV-positive and HPV-negative subgroups highlights enhanced coupling of the APOBEC3B—ASF1B and APOBEC3B—RPA2 pairs in HPV-positive tumors.(F) Predicted molecular interaction and signaling network of the APOBEC3B axis generated using STRING (https://string-db.org/). (G) Analysis of the GSE65858 cohort reveals that the downregulated cluster comprising MMPs, COL12A1, and TGFBI indicates suppression of the TGF-β—ECM remodeling/EMT pathway, potentially reflecting microenvironmental adaptation following treatment or within specific patient subgroups. **Abbreviations:** EMT, epithelial—mesenchymal transition; ECM, extracellular matrix; HNSCC, head and neck squamous cell carcinoma; STRING, search tool for the retrieval of interacting genes/proteins.

treatment or within specific subgroups. These correlations highlight a potential APOBEC3B—TGFB1 signaling axis that orchestrates the dual regulation of DNA repair and epithelial—mesenchymal transition (EMT) in HPV^{+} oropharyngeal cancer (OPC) (Fig. 2G). These results indicate that APOBEC3B and its downstream network are closely linked to replication-stress responses and EMT regulation in HPV-positive tumors, supporting its potential as a key modulator and biomarker in oropharyngeal cancer progression.

Explore the association between silencing APOBEC3B and related genes in vitro

We next investigated the functional consequences of APOBEC3B silencing on downstream signaling and malignant phenotypes in vitro. A panel of human head and neck squamous cell carcinoma (HNSCC) cell lines, encompassing both HPV-negative and HPV-positive types, was screened to identify appropriate models for functional validation (Fig. 3A). Based on APOBEC3B expression levels and radiation sensitivity, SCC-4 (HPV-negative) and SCC-154 (HPV-positive) cells were selected for further experiments. To generate irradiation-resistant derivatives, both lines were exposed to fractionated X-ray irradiation (2 Gy every 48 h for 30 cycles, total dose = 60 Gy) using an X-RAD 320 irradiator (Precision X-Ray, North Branford, CT, USA). Stable APOBEC3B-knockdown cells were established by lentiviral transduction of short-hairpin RNA (shAPOBEC3B; Thermo Fisher Scientific, USA) and validated by quantitative PCR (Fig. 3B). Silencing APOBEC3B in SCC-154-IR cells significantly reduced the mRNA expression of ASF1B and RPA2, indicating disruption of the APOBEC3B/ASF1B regulatory axis. Functional assays demonstrated that shAPOBEC3B cells exhibited markedly reduced migratory and invasive capacities, accompanied by increased sensitivity to cisplatin (5—20 μM; Sigma—Aldrich, St. Louis, MO, USA) as measured by CellTiter-Glo Luminescent Cell Viability

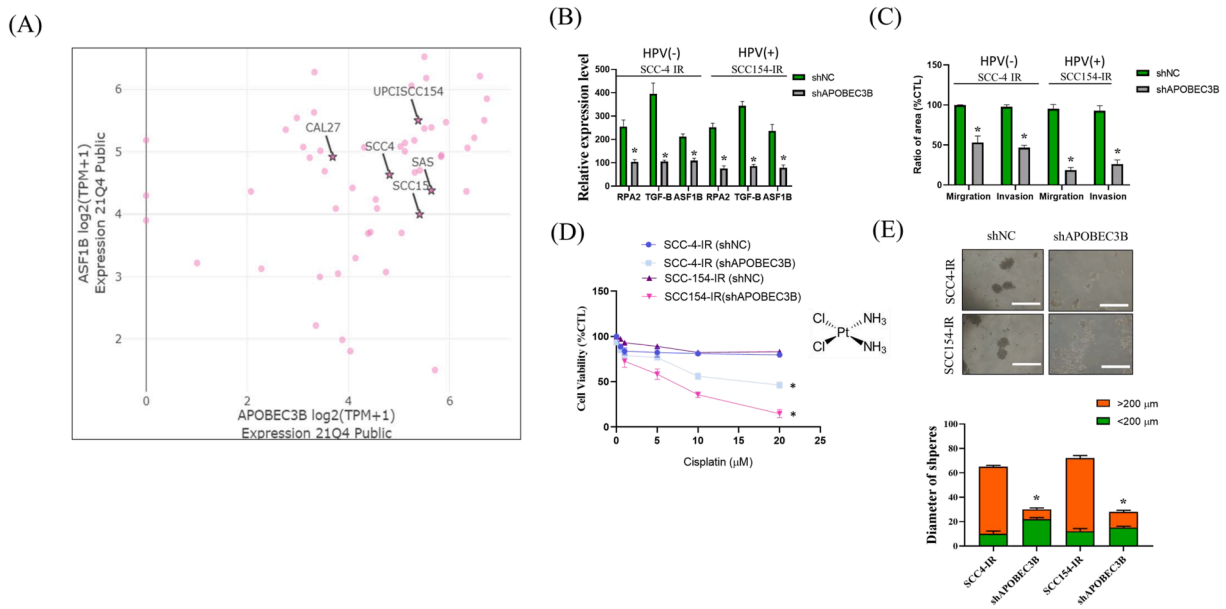


Figure 3 Functional characterization of APOBEC3B silencing and its downstream effects in vitro. (A) DepMap analysis identifying HPV-negative (SCC-4) and HPV-positive (SCC-154) oral squamous cell carcinoma lines with differential APOBEC3B expression suitable for functional validation. (B) Quantitative PCR analysis showing that APOBEC3B silencing significantly downregulates its downstream targets ASF1B and RPA2, indicating disruption of the APOBEC3B/ASF1B regulatory axis. (C—D) Cisplatin sensitivity, migration, and invasion assays demonstrate that APOBEC3B knockdown reduces cell motility and enhances cisplatin-induced cytotoxicity, as assessed by CellTiter-Glo viability assay (Promega) and Matrigel-based invasion analysis.(E) Tumor spheroid formation assay showing that shAPOBEC3B markedly suppresses both the number and size of tumor spheres in HPV-positive SCC-154 cells, indicating impaired self-renewal capacity and partial re-sensitization of irradiation-resistant cells to treatment.Data represent mean ± SD from three independent experiments; statistical significance was determined by Student's t-test ($P < 0.05$). **Abbreviations:** SD, standard deviation; EMT, epithelial—mesenchymal transition; HPV, human papillomavirus; shRNA, short hairpin RNA.

Assay (Promega, Madison, WI, USA) (Fig. 3C and D). These results suggest that APOBEC3B suppression diminishes both motility and chemoresistance, implicating its role in sustaining aggressive phenotypes in HPV-positive OSCC. Moreover, tumor spheroid formation was profoundly inhibited following APOBEC3B knockdown, with both the number and mean diameter of spheroids decreased by over 50 % compared with control cells (Fig. 3E), indicating that APOBEC3B supports self-renewal and stem-like features under anchorage-independent conditions. APOBEC3B knockdown downregulates ASF1B and RPA2, attenuates TGF-β pathway activation, and suppresses EMT-associated functions such as migration, invasion, drug resistance, and spheroid formation. These data underscore the pivotal role of the APOBEC3B/ASF1B axis in promoting tumor aggressiveness and adaptive therapy resistance in HPV-positive oropharyngeal carcinoma, revealing a mechanistic link between viral infection, replication stress, and malignant progression.

Discussion

Human papillomavirus (HPV) infection has long been recognized as a key etiologic factor in oropharyngeal squamous cell carcinoma (OPSCC), yet the molecular determinants that distinguish HPV-positive tumors from HPV-negative counterparts remain incompletely understood.[10,11] Among these, the cytidine deaminase APOBEC3B (A3B) has emerged as a crucial mutagenic enzyme that bridges viral infection, DNA damage, and tumor evolution. Although APOBEC3B normally functions as part of the intrinsic antiviral defense system, persistent HPV infection appears to hijack and dysregulate this enzyme, converting a host restriction factor into a mutational driver of oncogenesis.[12,13] Previous genomic studies have revealed that 98 % of HPV-positive HNSCCs exhibit APOBEC mutational signatures compared with approximately 76 % of HPV-negative cases, implicating APOBEC-mediated DNA editing as a predominant mutational process in HPV-associated tumors.[14] Unlike HPV-negative HNSCC, where tobacco-related mutagens dominate, HPV-positive tumors display mutation patterns characteristic of cytidine-to-uracil deamination events.[15] This observation suggests that viral infection not only induces APOBEC3B expression but also sustains its aberrant enzymatic activity within epithelial cells, thereby fueling genomic instability and clonal diversification.[16] APOBEC family members, including AID (Activation-Induced Cytidine Deaminase) and APOBEC3 enzymes, share the ability to convert cytosine to uracil in single-stranded DNA, generating mutations during DNA replication or repair.[17] While such mechanisms provide antiviral protection, their dysregulation in cancer leads to uncontrolled mutagenesis. Germline variants or

transcriptional activation of APOBEC3B have been associated with increased somatic mutation burden and cancer risk.[18] Moreover, several studies suggest that APOBEC dysregulation may enhance HPV replication and integration, further promoting tumor progression and metastatic spread.[19]

Our study extends these insights by identifying a functional connection between the APOBEC3B/ASF1B axis and the TGF-β signaling pathway in HPV-positive oropharyngeal carcinoma. Transcriptomic analysis of TCGA-HNSC datasets demonstrated coordinated overexpression of APOBEC3B, ASF1B, RPA2, and cell cycle—related genes such as MCM5 and TMPO in HPV-positive tumors. These findings were consistent with our own RNA sequencing data from clinically collected specimens. The enrichment of these replication-associated factors suggests that APOBEC3B activation may couple DNA replication stress with epithelial—mesenchymal transition (EMT), contributing to the invasive phenotype characteristic of HPV-positive OPSCC.

In vitro functional studies further substantiated this hypothesis. Silencing APOBEC3B in the HPV-positive SCC-154 cell line and its irradiation-resistant derivative (SCC-154-IR) led to significant downregulation of ASF1B and RPA2 expression, disruption of the TGF-β/Smad2/3 cascade, and suppression of EMT markers. Functionally, shAPOBEC3B cells exhibited reduced migratory and invasive abilities, increased cisplatin sensitivity, and markedly diminished tumor spheroid formation, indicating that APOBEC3B supports both self-renewal and therapy resistance. These findings provide direct experimental evidence that APOBEC3B overexpression drives aggressive, treatment-tolerant phenotypes in HPV-positive oropharyngeal cancer through modulation of replication and TGF-β signaling pathways. The interplay between APOBEC3B and ASF1B, a histone chaperone involved in chromatin assembly and DNA replication, appears to be particularly important. Prior studies in cervical and gastric cancers have shown that ASF1B upregulation enhances proliferation, migration, and cisplatin resistance via MYC activation, while ASF1B silencing induces apoptosis and sensitizes cells to chemotherapy.[20] In our model, APOBEC3B silencing produced parallel effects—reducing ASF1B and RPA2 levels, inhibiting TGF-β—mediated EMT, and restoring drug responsiveness—suggesting that these two molecules act in concert to promote oncogenic adaptation. Our results delineate a mechanistic framework in which HPV infection induces APOBEC3B overexpression, which in turn activates the ASF1B—RPA2—TGF-β axis, leading to enhanced cell proliferation, EMT, and resistance to therapy. This APOBEC3B-driven feedback loop may underlie the paradoxical combination of high mutational load and relatively good prognosis observed in HPV-positive OPSCC: while APOBEC3B promotes tumor evolution and heterogeneity, it may also increase immunogenicity, rendering tumors more responsive to chemoradiation and immune checkpoint therapy.[21] Our study identifies the APOBEC3B/ASF1B—TGF-β signaling pathway as a key molecular network in HPV-positive oropharyngeal carcinoma. Silencing APOBEC3B not only suppresses proliferation, migration, and spheroid formation but also restores cisplatin sensitivity, underscoring its potential as a therapeutic target. Future work should focus on developing selective APOBEC3B inhibitors and exploring combinatorial strategies with radiotherapy or immune checkpoint blockade to overcome resistance and improve patient outcomes.[22]

Clinically, HPV-induced oropharyngeal cancer generally exhibits a more favorable prognosis and is preventable through vaccination. However, a subset of HPV-positive patients eventually develop radio- and chemoresistance, often followed by distant metastasis and disease recurrence, leading to poor clinical outcomes. Our present findings demonstrate that APOBEC3B is markedly overexpressed in human cancer tissues compared with normal mucosa, supporting its role in tumor progression and therapeutic resistance. Future research will focus on elucidating the mechanistic regulation of the APOBEC3B/ASF1B axis in radioresistant oropharyngeal cancer, particularly its interaction with DNA damage response and TGF-β signaling

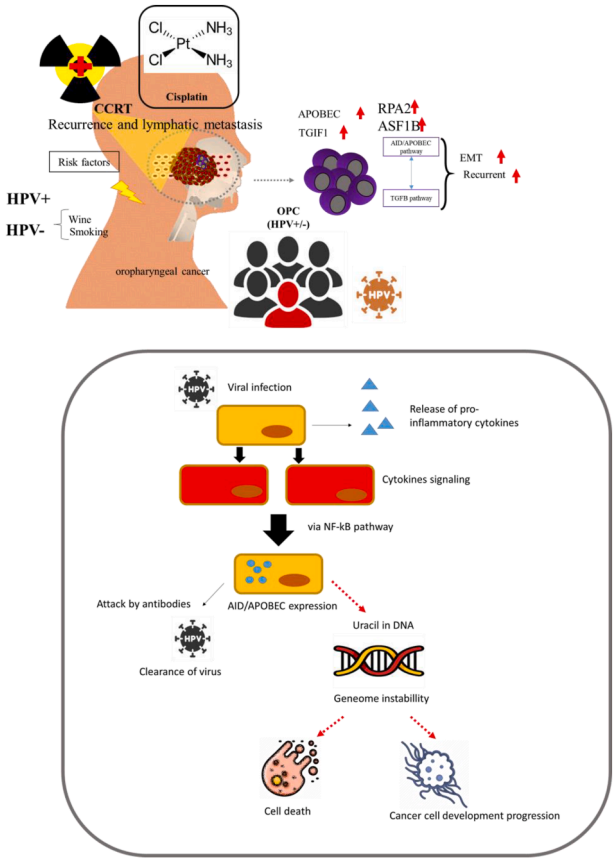


Figure 4 Graphical summary and proposed mechanism of APOBEC3B—TGF-β1 signaling in HPV-positive oropharyngeal carcinoma. Schematic illustration summarizing the findings of this study. Clinical specimen analysis and TCGA data reveal abnormal co-expression of APOBEC3B and TGF-β1 in HPV-positive oropharyngeal carcinoma. HPV infection induces APOBEC3B expression in oropharyngeal epithelial cells, which activates TGF-β signaling, leading to RPA2/ASF1B-mediated proliferation, EMT, and therapy resistance. **Abbreviations:** EMT, epithelial—mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; TCGA, The Cancer Genome Atlas; TGF-β, transforming growth factor-β.

pathways. In parallel, we aim to determine whether APOBEC3B/ASF1B activity is essential for maintaining cancer cell metabolism, stemness, and the tumor microenvironment, which may collectively contribute to treatment resistance. By defining how HPV infection reprograms this pathway, we hope to identify novel therapeutic vulnerabilities and develop APOBEC3B/ASF1B-targeted strategies to overcome resistance and improve clinical outcomes in HPV-positive oropharyngeal carcinoma. A schematic representation of our proposed model is illustrated in Fig. 4.

Declaration of competing interest

The authors confirm that they do not have any potential financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2025.10.039.

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