Mechanism of action of *Aloe vera* against human papillomavirus-positive head and neck squamous cell carcinoma

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Abstract. The present study aimed to evaluate the therapeutic targets of *Aloe vera* against human papillomavirus-positive (HPV$^+$) head and neck squamous cell carcinoma (HNSCC), focusing on key bioactive components and their impact on molecular pathways, gene expression, overall survival and the tumor immune microenvironment. Using network pharmacology analysis, eight bioactive components of *Aloe vera* were identified, along with 244 corresponding potential therapeutic targets. Differential gene expression analysis of the GSE53355 and GSE117973 datasets revealed HPV$^+$ HNSCC-specific genes. Using topological analysis of protein-protein interaction networks, serpin family member 1 (SERPINE1) was determined to be the core gene. Gene expression data were validated and survival analysis was performed, alongside molecular docking and experimental validation in HPV$^+$ HNSCC cells. SERPINE1 was identified as a key gene that was markedly upregulated in HPV$^+$ HNSCC, with a strong association with worse overall survival (P<0.0001). Genetic alterations in SERPINE1, primarily arm-level gains, were associated with immune cell infiltration, suggesting a role in tumorigenesis. Gene Set Enrichment Analysis showed the involvement of SERPINE1 in crucial cancer-associated pathways. Quercetin and SERPINE1 have strong binding affinity, as demonstrated by molecular docking, with experimental validation showing dose-dependent downregulation of SERPINE1 in HPV$^+$ HNSCC cells. In conclusion, the bioactive component of *Aloe vera*, quercetin, exhibits potential in specifically targeting SERPINE1. The present discovery offers a potential therapeutic strategy for HPV$^+$ HNSCC, particularly for enhancing patient survival outcomes. However, further in-depth investigations are essential to validate its therapeutic efficacy and underlying mechanisms within the intricate tumor microenvironment *in vivo*.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) represents the most prevalent form of cancer in the head and neck area, accounting for >90% of cases, with a 5-year overall survival rate of 50-60% (1). Human papillomavirus (HPV) serves as a primary risk-contributing factor for HNSCC, contributing to ~70% of HNSCC cases in developed nations (2,3). The pathogenesis and progression of HPV$^+$ HNSCC involve intricate molecular regulatory mechanisms and signaling pathways, such as the regulation of apoptosis, cell cycle progression and the immune response through viral oncogenes or epigenetic silencing, leading to notable differences in clinical characteristics, treatment responses and prognosis compared with HPV$^-$ HNSCC (4,5). Currently, there are no specific therapies for HPV$^+$ HNSCC, which is still treated using standard HNSCC strategies such as surgery, radiation and chemotherapy. These traditional approaches frequently give rise to substantial side effects, including toxicity, as well as malformation and dysfunction caused by partial or complete resection of the jawbone or other maxillofacial organs (6). Moreover, the clinical application and efficacy of immunotherapies are restricted, making it imperative to identify effective therapeutic targets and treatments for HPV$^+$ HNSCC (7).

Traditional Chinese medicine (TCM), notable for its low toxicity, multi-component nature and multi-target effects, has become a promising therapeutic option (8). With progress in network pharmacology, exploring the components and

mechanisms of TCM for anticancer effects has become more efficient. For example, a study on Yinchen Wuling San identified key compounds, such as cerevisterol, polyporusterone E and genkwanin, which may exert anti-HNSCC effects through pathways such as PI3K-Akt and MAPK signaling (9). This research underscores the potential of TCM as a valuable source for developing novel anticancer drugs.

Aloe vera (L.) Burm.f., a perennial succulent herb, is widely used in TCM for its medicinal properties, including immune modulation, anti-inflammatory, antiviral and hypoglycemic effects (10,11). Previously, its antitumor efficacy has gained attention, demonstrating activity against various types of cancer. For example, aloe-emodin, a bioactive compound found in *Aloe vera*, can trigger apoptosis in breast and lung cancer by disrupting mitochondrial function (12). Additionally, ethanolic leaf extracts of *Aloe vera* exhibit cytotoxic effects against cervical cancer and lung adenocarcinoma cells (13). The anticancer effects of several therapeutic ingredients have been identified, including anthraquinones, *Aloe vera* polysaccharides and aloctin (14); however, the therapeutic effects of *Aloe vera* on HPV+ HNSCC remain unclear.

Previous studies have underscored the potential of serpin family member 1 (SERPINE1) as a promising target in HNSCC therapy, particularly due to its role in regulating tumor progression, metastasis and immune evasion (15,16). In the context of HPV- HNSCC, emerging evidence has indicated that SERPINE1 serves a critical role in modulating tumor microenvironment dynamics and immune responses (17). Additionally, studies have demonstrated that the expression of SERPINE1 is regulated by multiple mechanisms, including m6A modifications mediated by the α-ketoglutarate-dependent dioxygenase fat mass and obesity-associated protein and circular RNAs, such as circPRMT5, which stabilize SERPINE1 mRNA through interactions with insulin-like growth factor 2 mRNA-binding protein 3 (15,18). These findings collectively suggest that targeting SERPINE1 could offer a novel therapeutic strategy for intervening in tumor biology and enhancing antitumor immunity.

Based on network pharmacology analysis, the present study aimed to identify potential therapeutic ingredients and targets of *Aloe vera* against HPV+ HNSCC. Hub genes were screened through topological analysis of protein-protein interaction (PPI) networks and validated using The Cancer Genome Atlas (TCGA) data for expression levels and prognostic importance. Molecular docking was utilized to investigate the effective ingredients and key targets. Subsequently, the antitumor effects were evaluated through western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The research aimed to provide insights for the development of anti-HPV+ HNSCC drugs derived from TCM.

Materials and methods

Ingredients and targets of Aloe vera. The active ingredients and associated targets of *Aloe vera* were explored through TCM Systems Pharmacology Database and Analysis Platform (TCMSP; https://old.tcmsp-e.com/index.php) and SwissTargetPrediction (http://www.swisstargetprediction.ch/) platforms. The interaction between ingredients and targets was established using Cytoscape v3.10.2 software (https://cytoscape.org/). A threshold of 30% for the oral bioavailability and 0.18 for the drug likeness was set for TCMSP, with a probability score of 1 for SwissTargetPrediction (19,20).

Differential gene expression analysis of HPV+ HNSCC. GSE53355 and GSE117973 were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) using the key words 'HPV positive HNSCC', with GSE53355 containing 44 samples (26 HPV+ HNSCC, 18 HPV- HNSCC) and GSE117973 comprising 77 samples (54 HPV+ HNSCC, 23 HPV- HNSCC) (21,22). Differential gene expression analysis was performed using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) ($P<0.05$; logFC >1).

Identification of potential targets of Aloe vera against HPV+ HNSCC. A Venn diagram was employed to analyze the overlap between the predicted genes of *Aloe vera* and those associated with HPV+ HNSCC, thereby identifying potential therapeutic targets for this cancer subtype.

Functional enrichment analysis. Functional enrichment analysis was performed employing WebGestalt v2024 (http://www.webgestalt.org). Top-ranked Gene Ontology (GO) terms including biological processes (BPs), molecular functions (MFs) and cellular components (CCs), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were visualized as bar charts and bubble plots (23). The reference gene set was set to all genome protein-coding genes, to serve as the background for enrichment calculations.

Construction of a PPI network and hub gene identification. PPI networks based on common targets between *Aloe vera* and HPV+ HNSCC were created using the STRING v12.0 platform (https://cn.string-db.org/) and were visualized in Cytoscape. MCODE v2.0.3 (https://apps.cytoscape.org/apps/mcode) and Network Analyzer plugins were employed to calculate the MCODE score, betweenness centrality (BC), closeness centrality (CC) and degree for each node, with the top 10 genes identified as hub genes (24).

Expression validation and prognostic analysis. The prognostic relevance of hub genes in a cohort of patients with HNSCC (n=500) was analyzed using the Kaplan-Meier Plotter (https://kmplot.com/analysis/) to evaluate overall survival outcomes, employing the automatic selection of the optimal cutoff based on percentile. To ensure the robustness of the findings, the results of the survival analysis were further validated using the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) and UALCAN (https://ualcan.path.uab.edu/) platforms. These platforms offer valuable insights into the association between gene expression and survival based on TCGA datasets. Significant associations were determined using a threshold of $P<0.05$.

Genomic alterations and immune infiltrations. The cBioPortal (www.cbioportal.org/) and TIMER2.0 (http://timer.comp-genomics.org/) were employed to examine the somatic copy number alterations (SCNAs) in key *Aloe vera* targets against HPV+ HNSCC, and the correlation between SCNAs and immune cell infiltrations. The infiltration levels for each

SCNA category were compared with normal controls using a two-sided Wilcoxon rank-sum test.

Co-expression analysis and Gene Set Enrichment Analysis (GSEA). The LinkedOmics platform (www.linkedomics.org/) was employed to investigate the co-expression of the hub gene, alongside GSEA to investigate their functional implications (25).

Molecular docking. The core pharmacological compounds of *Aloe vera*, along with the protein structures of the key genes, were sourced from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) and Protein Data Bank (PDB; https://www.rcsb.org/) databases. Molecular docking was performed using PyRx software v0.8 (https://pyrx.sourceforge.io/) that was integrated with AutoDock Vina v1.2.x (https://vina.scripps.edu/). This process identified critical molecular interactions, with a binding energy threshold of 7 kcal/mol.

Experimental validation. Quercetin (Shanghai Yuanye Biotechnology Co., Ltd.) was dissolved in dimethyl sulfoxide (MilliporeSigma) to create solutions with concentrations of 5 and 10 μM. Post-sonication (30 min, 40 kHz, 25˚C) and centrifugation (5 min, 13,500 x g, 4˚C), the supernatant was collected. Equivalent concentrations of dimethyl sulfoxide without quercetin were used as a control.

Cell culture. UPCI:SCC154 cells (CRL-3241; American Type Culture Collection) were cultured in DMEM supplemented with 10% FBS and 100 U/ml penicillin/streptomycin (all Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in 5% CO_2.

Western blotting. After being treated for 24 h, total protein was extracted using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology) containing protease and phosphatase inhibitors. Protein concentration was determined using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). Equal amounts of protein (30 μg per lane) were then separated by SDS-PAGE on 10% gels and transferred to polyvinylidene fluoride membranes (Thermo Fisher Scientific, Inc.). The membranes were blocked with 5% non-fat milk in TBS-0.1% Tween-20 (TBST) for 1 h at room temperature, and then incubated overnight at 4˚C with the following primary antibodies: Anti-SERPINE1 (1:1,000 dilution; cat. no. 13801-1-AP; Proteintech Group, Inc.) and anti-β-actin (1:5,000 dilution; cat. no. 66009-1-Ig; Proteintech Group, Inc.). After washing three times with TBST, the membranes were incubated with HRP-conjugated secondary antibodies (goat anti-rabbit IgG, 1:5,000, cat. no. SA00001-2; goat anti-mouse IgG, 1:5,000, cat. no. SA00001-1; both from Proteintech Group, Inc.) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection kit (MilliporeSigma), and imaged using a ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Inc.) Semi-quantification of protein bands was performed using ImageJ software v1.54 (National Institutes of Health).

RT-qPCR. Cells were treated for 24 h before RNA extraction. Total RNA was extracted using RNAiso Plus reagent (Takara Bio Inc.), following the manufacturer's instructions. RT was performed using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Bio Inc.) according to the manufacturer's protocol. qPCR was conducted using TB Green® Premix Ex Taq™ II (Takara Bio Inc.,) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used were as follows: SERPINE1: forward 5'-ACCGCAACGTGGTTTTCTCA-3', reverse 5'-TTGAATCCCATAGCTGCTTGAAT-3'; and β-actin: forward 5'-AGAGCTACGAGCTGCCTGAC-3', reverse 5'-GTCACCTTCACCGTTCCAGT-3'.

The qPCR cycling protocol included an initial denaturation step at 95˚C for 3 min, followed by 35 cycles of denaturation at 95˚C for 20 sec, annealing at 56˚C for 20 sec and extension at 72˚C for 20 sec. Melting curve analysis was performed with the following steps: 95˚C for 15 sec, 60˚C for 15 sec, 95˚C for 20 min and 95˚C for 15 sec. All reactions were performed in triplicate. Relative gene expression was quantified using the $2^{-\Delta\Delta Cq}$ method, with β-actin used as the internal control (26).

Statistical analysis. The experimental data were statistically analyzed using GraphPad Prism v6.0 software (Dotmatics). Differences between two groups were assessed using an unpaired Student's t-test, while multi-group comparisons were performed using a one-way ANOVA followed by Dunnett's test for post hoc analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Targets of Aloe vera against HPV⁺ HNSCC. Using network pharmacology analysis, eight key bioactive components of *Aloe vera* were identified: Aloe-emodin, aloeresin C, arachidonic acid, β-carotene, campest-5-en-3β-ol, cholesterol, quercetin and sitosterol (Fig. 1A). Concurrently, 244 potential therapeutic targets for *Aloe vera* were identified (Fig. 1B). Differential gene expression analysis of the GSE53355 dataset revealed 402 HPV⁺ HNSCC-specific genes, including 257 upregulated and 145 downregulated genes (Fig. 2A). From the GSE117973 dataset, 461 HPV⁺ HNSCC-specific genes were detected (194 upregulated and 267 downregulated; Fig. 2B). The integrated analysis of two datasets identified a total of 768 genes specifically associated with HPV⁺ HNSCC. Through the intersection of *Aloe vera* targets and HPV⁺ HNSCC-specific genes, 34 potential therapeutic targets were identified as targets for *Aloe vera* against HNSCC(Fig. 2C) and a drug-target-disease network was constructed (Fig. 2D).

Functional enrichment analyses. GO analysis indicated that the target genes primarily participated in BPs such as 'response to lipid' and 'response to oxygen-containing compound' (Fig. 3A and Table SI). At the MF level, they were associated with 'bile acid binding' and 'estradiol 17-beta-dehydrogenase [NAD(P)] activity' and were localized in the 'extracellular matrix' and 'external encapsulating structure'. KEGG pathway analysis revealed their participation in pathways such as 'AGE-RAGE signaling pathway in diabetic complications' and 'IL-17 signaling pathway' (Fig. 3B).

Topological analysis and identification of hub genes. A PPI network comprising 29 nodes was created using STRING and Cytoscape (Fig. 4A). A total of two significant clusters within the network were identified by employing the MCODE plugin. By integrating top-ranking nodes based on centrality measures

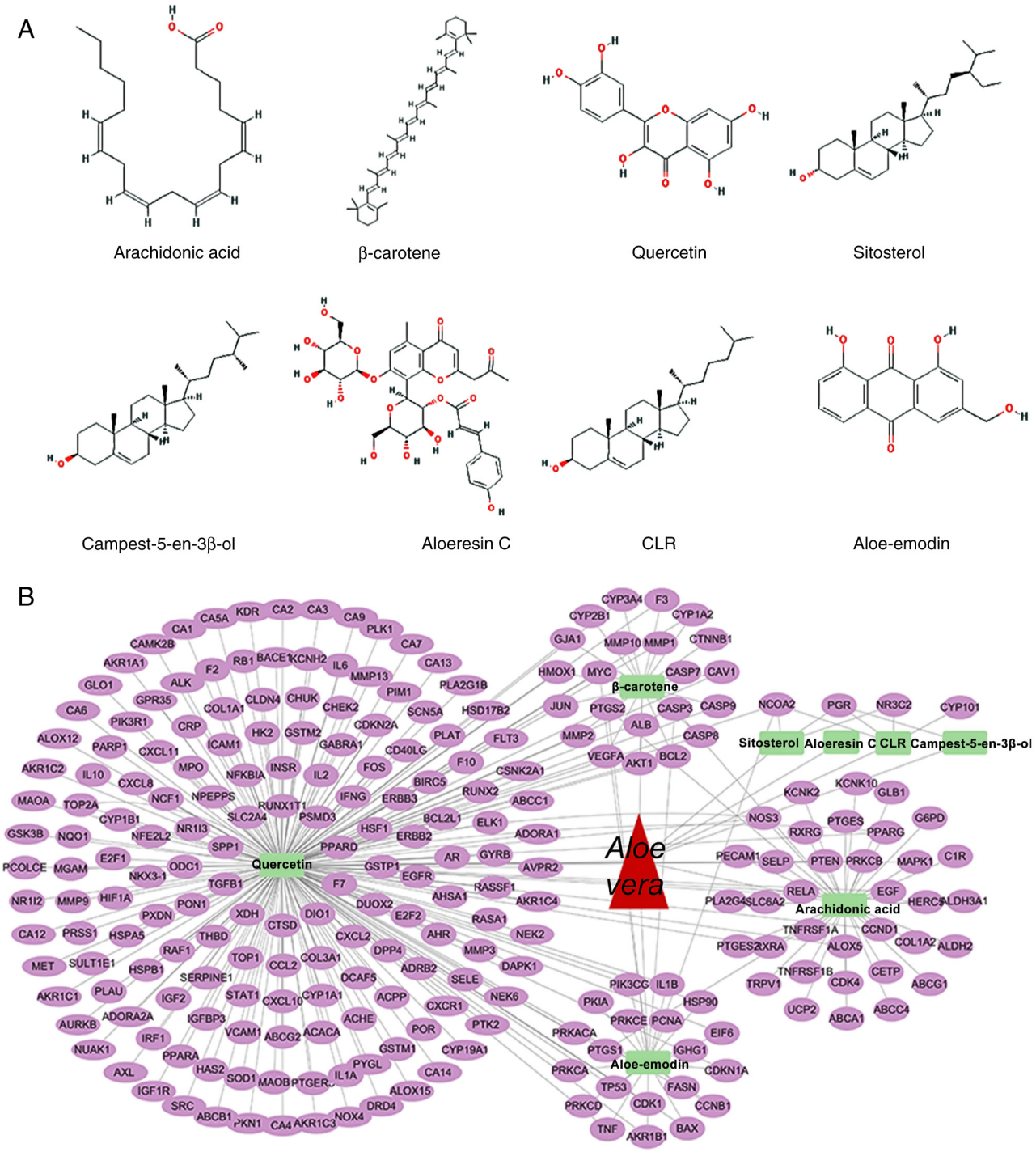


Figure 1. Analysis of therapeutic ingredients and targets of *Aloe vera*. (A) Structures of eight medicinal components of *Aloe vera*. (B) Network showing the relationship between *Aloe vera* ingredients and their targets. Red represents *Aloe vera*, green represents the eight therapeutic ingredients and purple indicates potential targets.

such as degree, BC, CC and MCODE scores, PTGS2, IL6, IL1B, CXCL8 and SERPINE1 were recognized as the hub genes (Fig. 4B and C).

Validation of hub gene expression and prognostic relevance. SERPINE1 expression was markedly upregulated in HPV+ HNSCC tissues compared with that in normal tissues from healthy individuals (Fig. 5A) and was increased across all stages of HNSCC (Fig. 5B), as well as in eight other types of cancer (Fig. 5C). Survival analysis revealed that SERPINE1 expression was significantly associated with overall survival in patients with HNSCC (Fig. 5D).

Genetic alterations and immune infiltrations. The overall frequency of genetic alterations in SERPINE1 in HNSCC was ~5%, suggesting a potential genetic predisposition associated with this gene (Fig. 6A). Amplifications were particularly common and constituted a substantial proportion

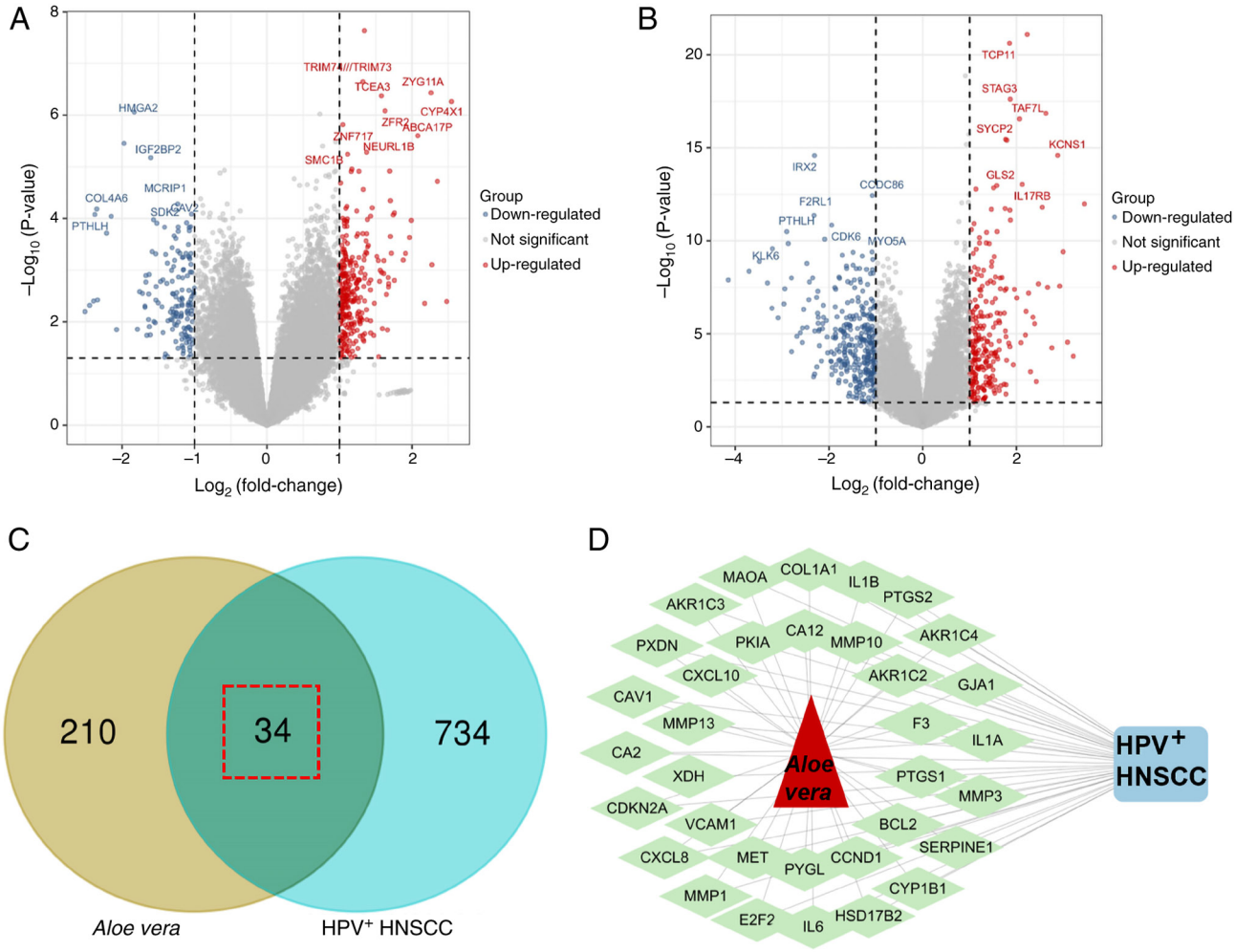


Figure 2. Analysis of shared target genes between *Aloe vera* and HPV+ HNSCC. Differential gene expression analysis was performed on (A) GSE53355 and (B) GSE117973 datasets to obtain volcano plots of target genes in HPV+ HNSCC. (C) Intersection of target genes between *Aloe vera* and HPV+ HNSCC yielded a total of 34 shared genes, visualized using a Venn diagram. (D) A drug-target-disease network was constructed with 34 shared genes between *Aloe vera* and HPV+ HNSCC. HPV, human papillomavirus; HNSCC, head and neck squamous cell carcinoma.

of these alterations. By contrast, missense and truncating mutations occurred at a lower frequency. In HPV+ HNSCC, SERPINE1 frequently underwent arm-level gains and deletions, suggesting that gene dosage alterations may contribute to tumorigenesis in this subgroup (Fig. 6B). These gains were associated with the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells (Fig. 7A). Moreover, the increased expression of SERPINE1 was significantly correlated with reduced B-cell infiltration, which in turn was associated with worse survival outcomes in patients with HPV+ HNSCC (Fig. 7B and C). High levels of SERPINE1 expression were also associated with diminished overall survival in patients with HPV+ HNSCC.

Co-expression analysis and GSEA of SERPINE1 in HNSCC. Co-expression analysis of SERPINE1 in HNSCC revealed a significant association with several genes, including TGA5, INHBA, ACTN1, E2F2 and PDIK1L (Fig. 8A). Heatmaps showed the top 100 genes associated with SERPINE1, suggesting potential regulatory networks in HNSCC (Fig. 8B and C). Furthermore, GSEA revealed that key BPs were significantly associated with SERPINE1, including 'focal adhesion', 'ECM-receptor interaction' and 'PI3K-Akt signaling', all crucial for cell-matrix interactions and survival signaling (Fig. 8D-F). Additionally, pathways associated with 'human papillomavirus infection', 'base excision repair' and 'DNA replication' were significantly enriched, highlighting the potential role of SERPINE1 in the oncogenic mechanisms of HPV+ HNSCC (Fig. 8G-I).

Molecular docking of quercetin with SERPINE1 protein. Based on the results of network pharmacological analysis and the drug-component-target diagram (Fig. 1B), quercetin was identified as the primary bioactive component in *Aloe vera* that specifically targets SERPINE1. Subsequent molecular docking analysis revealed a strong binding interaction between quercetin and the SERPINE1 protein (PDB identification, 1LJ5), with a binding energy calculated at -7.1 kcal/mol (Fig. 9). The analysis revealed key interactions between quercetin and critical residues within the SERPINE1 active site, including hydrogen bonds with GLU378 and hydrophobic interactions involving VAL274, ALA239 and ILE237. These interactions suggested that quercetin may effectively inhibit the function of SERPINE1 by stabilizing its active conformation.

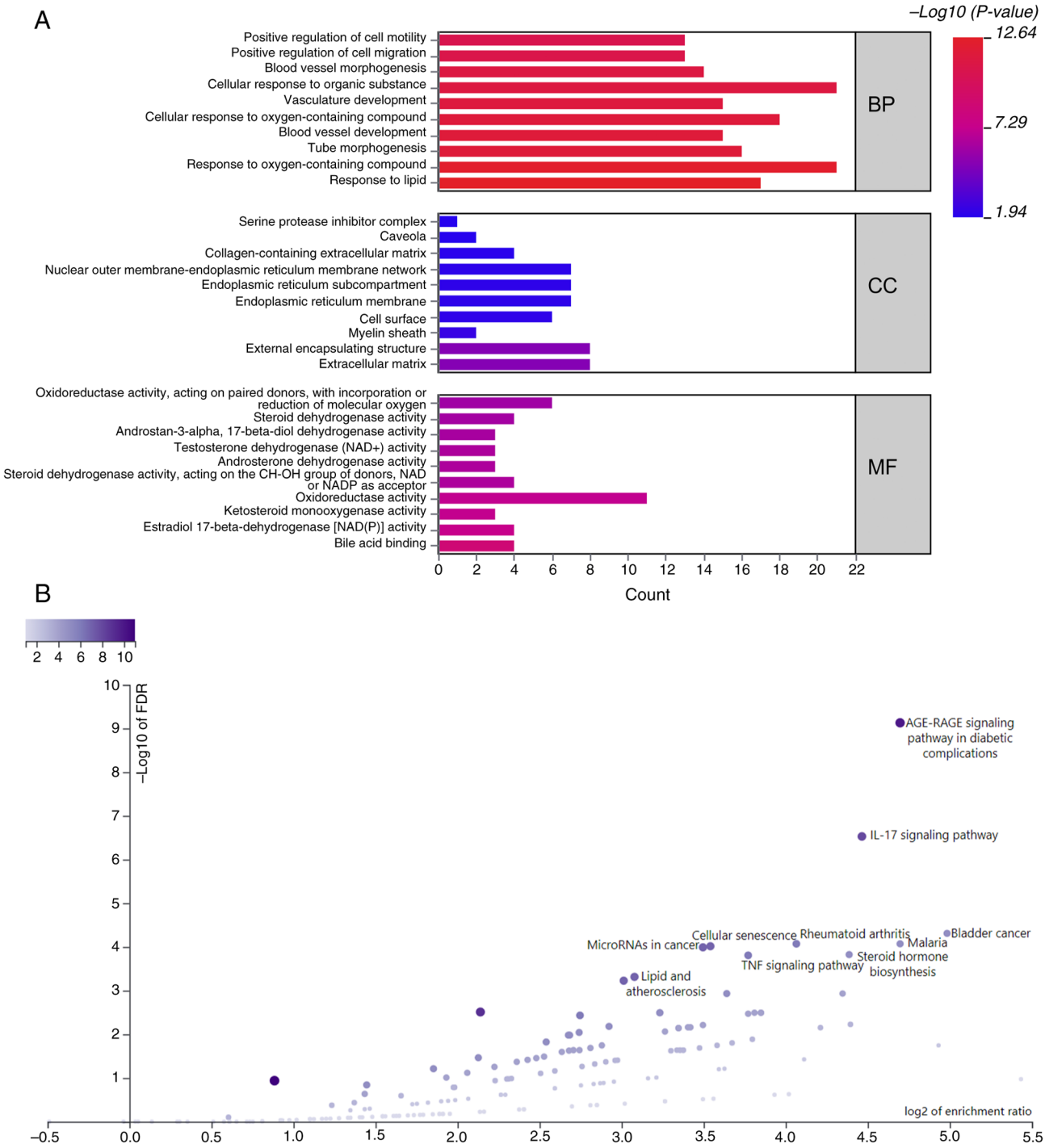
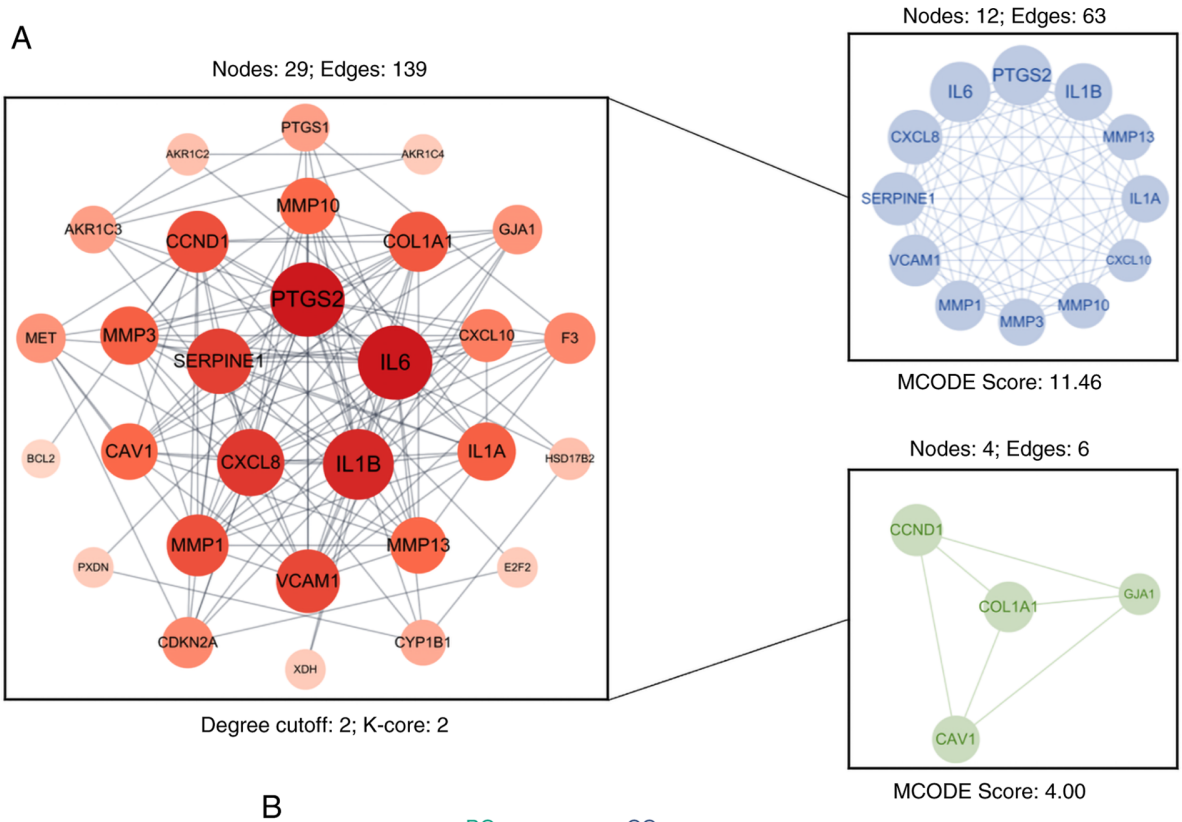


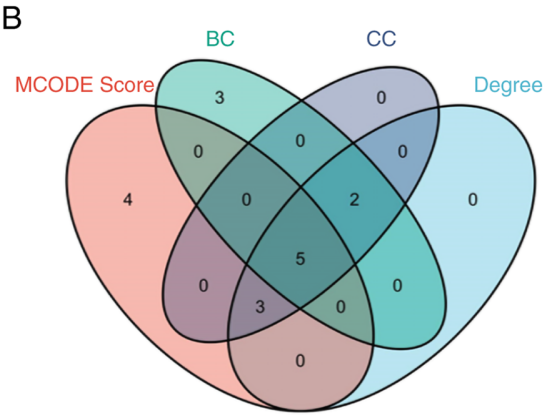
Figure 3. GO and Kyoto Encyclopedia of Genes and Genomes analysis results of *Aloe vera* and human papillomavirus-positive head and neck squamous cell carcinoma shared target genes. (A) Most significant GO terms associated with target genes. (B) Pathways such as 'AGE-RAGE signaling pathway in diabetic complications' and 'IL-17 signaling pathway' were identified. GO, Gene Ontology; FDR, false discovery rate.

Downregulation of SERPINE1 by quercetin in HPV+HNSCC Cells. The impact of quercetin on SERPINE1 expression was further evaluated in HPV+ HNSCC cells. A dose-dependent reduction in SERPINE1 expression was observed following quercetin treatment. At the mRNA level, a significant decrease was detected in response to 5 and 10 μM quercetin compared with 0 μM, with the effect being more pronounced at 10 μM (Fig. 10A). This decrease in mRNA levels was further validated by western blot analysis. This analysis confirmed that the protein levels of SERPINE1 were downregulated following treatment with quercetin, as depicted in Fig. 10B.

Discussion

HNSCC remains challenging to treat despite advancements in therapeutic strategies, primarily due to its complex molecular landscape. However, TCM has emerged as a potential therapeutic option for this malignancy. A previous study revealed that patients with oral cancer who received treatments with





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MCODE Score	Gene	Degree	Gene	BC	Gene	CC	Gene
9	CXCL10	21	PTGS2	0.25	PTGS2	0.80	PTGS2
9	MMP13	21	IL6	0.15	AKR1C3	0.76	IL6
8.84	MMP3	19	IL1B	0.13	IL6	0.70	IL1B
8.59	CXCL8	17	CXCL8	0.12	CCND1	0.65	CXCL8
8.59	MMP10	16	SERPINE1	0.06	IL1B	0.65	SERPINE1
8.59	IL1A	15	VCAM1	0.06	CYP1B1	0.62	CCND1
8.31	VCAM1	14	MMP1	0.06	COL1A1	0.62	MMP1
8.31	MMP1	14	CCND1	0.03	CXCL8	0.61	COL1A1
7.63	SERPINE1	13	COL1A1	0.02	CDKN2A	0.61	VCAM1
7.63	PTGS2	12	MMP3	0.02	SERPINE1	0.60	MMP3
7.63	IL6						
7.63	IL1B						

Figure 4. PPI network analysis. (A) A PPI network of *Aloe vera*- and human papillomavirus-positive head and neck squamous cell carcinoma shared targets was constructed, with two core subnetworks identified with degree cutoff=2 and K-core=2. (B) Based on MCODE score, BC, CC and degree, a total of five hub genes were identified, as shown in the Venn diagram. (C) Five hub genes were selected including PTGS2, IL6, IL1B, CXCL8 and SERPINE1, through the intersection of four gene sets. PPI, protein-protein interaction; BC, betweenness centrality; CC, closeness centrality; SERPINE1, serpin family member 1.

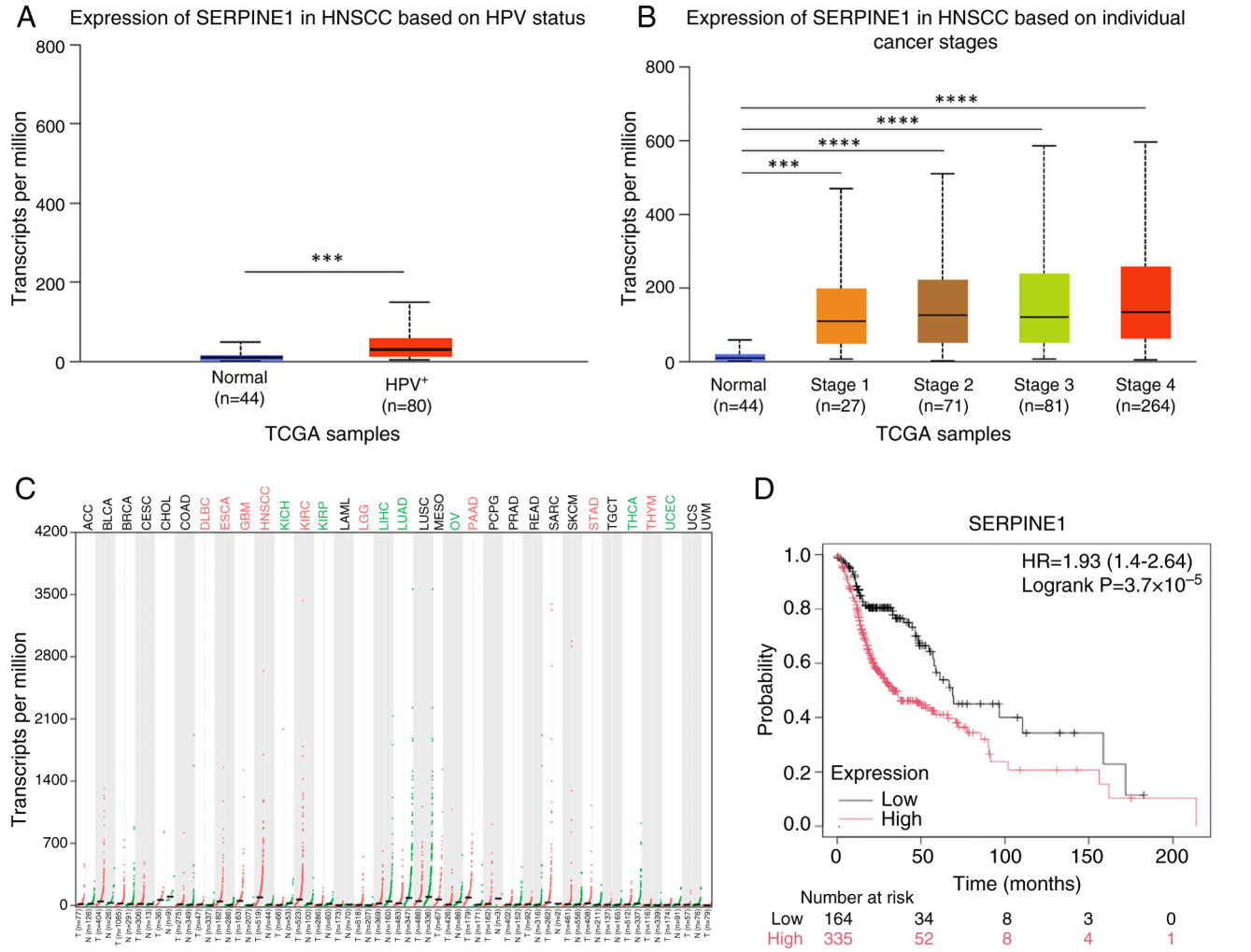

Figure 5. Expression of SERPINE1 and its prognostic implications in HNSCC. (A) Significantly elevated expression levels of SERPINE1 were observed in HPV+ HNSCC (***P<0.001). (B) SERPINE1 expression levels were significantly higher across different stages in HNSCC (***P<0.001; ****P<0.0001). (C) Elevated SERPINE1 levels were noted in HNSCC, as well as in eight additional cancer types (P<0.05). Colors indicate tumor expression differences: Red indicates upregulation, green indicates downregulation and black indicates no significant difference. (D) Kaplan-Meier curve illustrating the inverse association between SERPINE1 expression and overall survival. SERPINE1, serpin family member 1; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; TCGA, The Cancer Genome Atlas.

TCM formulations, such as Xuán shēn, Shí hú, Mài mén dōng and other herbal remedies, experienced a notable improvement in their overall survival rates (6). Further studies have suggested that TCM exerts antitumor effects by triggering apoptosis and by regulating the programmed death-1/programmed death-ligand 1 axis within the tumor immune microenvironment (TIME), thereby enhancing CD8+ T-cell activity and improving antitumor immunity (27,28).

Among various herbal formulations in TCM, *Aloe vera* has shown notable anticancer potential. In the present study, quercetin, a major bioactive compound in *Aloe vera*, demonstrated promising molecular interactions with SERPINE1. Quercetin (3,3',4',5,7-pentahydroxyflavone), broadly recognized for its immune-protective, anti-inflammatory and anti-aging effects, is a type of flavonol (29). Its antiviral action is also notable, particularly in its ability to bind to the HPV oncoprotein E6, interfering with the interaction between E6 and E6-associated protein, leading to G_2 phase arrest and apoptosis (30). Furthermore, quercetin demonstrates extensive anticancer potential by suppressing key signaling pathways such as mTOR and MAPK, increasing reactive oxygen species levels and regulating epithelial-mesenchymal transition markers together with matrix metalloproteinases (31-34). These actions effectively inhibit the migration, invasion and development of drug resistance in cancer cells. Additionally, its interaction with key proteins such as SERPINE1 indicates a targeted mechanism that holds promise for the treatment of HPV+ HNSCC.

Previous research has indicated that the upregulation of SERPINE1 is a marker of poor prognosis in several types of cancer, including HPV+ oral squamous cell carcinoma and gastric cancer (35,36). Additionally, SERPINE1 serves a vital role in cancer-associated pathways, such as ECM-receptor interaction and the PI3K-Akt signaling pathway, which are essential for cancer cell survival, migration and invasion. In HNSCC, SERPINE1 has also been associated with the infiltration of immune cells such as CD8+ T cells, CD4+ T cells and macrophages, thereby reshaping the TIME (37,38).

GSEA demonstrated an association between SERPINE1 and HPV infection in the present study. In addition, survival analysis highlighted SERPINE1 as a crucial gene

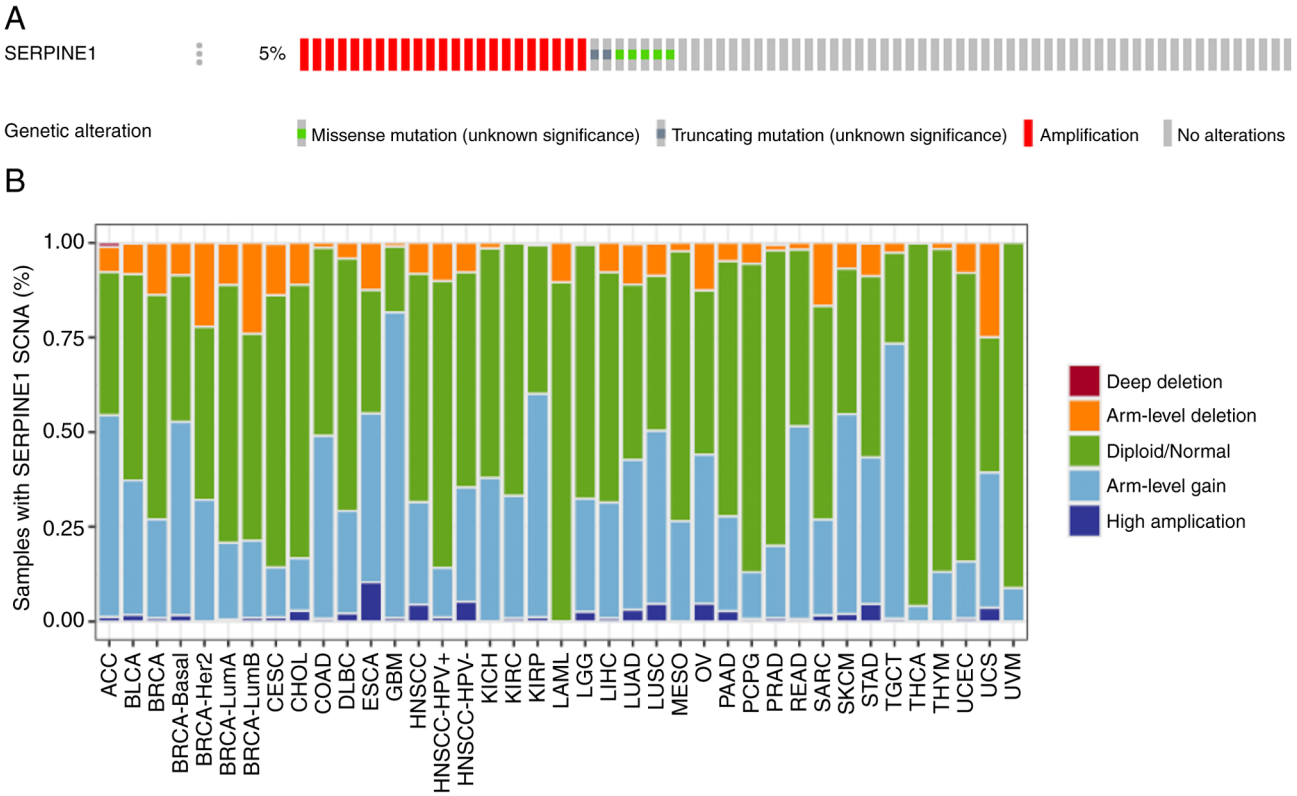

Figure 6. Genetic alterations and SCNAs of SERPINE1 in HPV⁺ HNSCC. (A) Genetic alteration profile of SERPINE1 in HNSCC, including missense mutations, truncating mutations and amplifications. (B) SCNAs profile of SERPINE1 across various types of cancer. SCNAs, somatic copy number alterations; SERPINE1, serpin family member 1; HPV, human papillomavirus; HNSCC, head and neck squamous cell carcinoma.

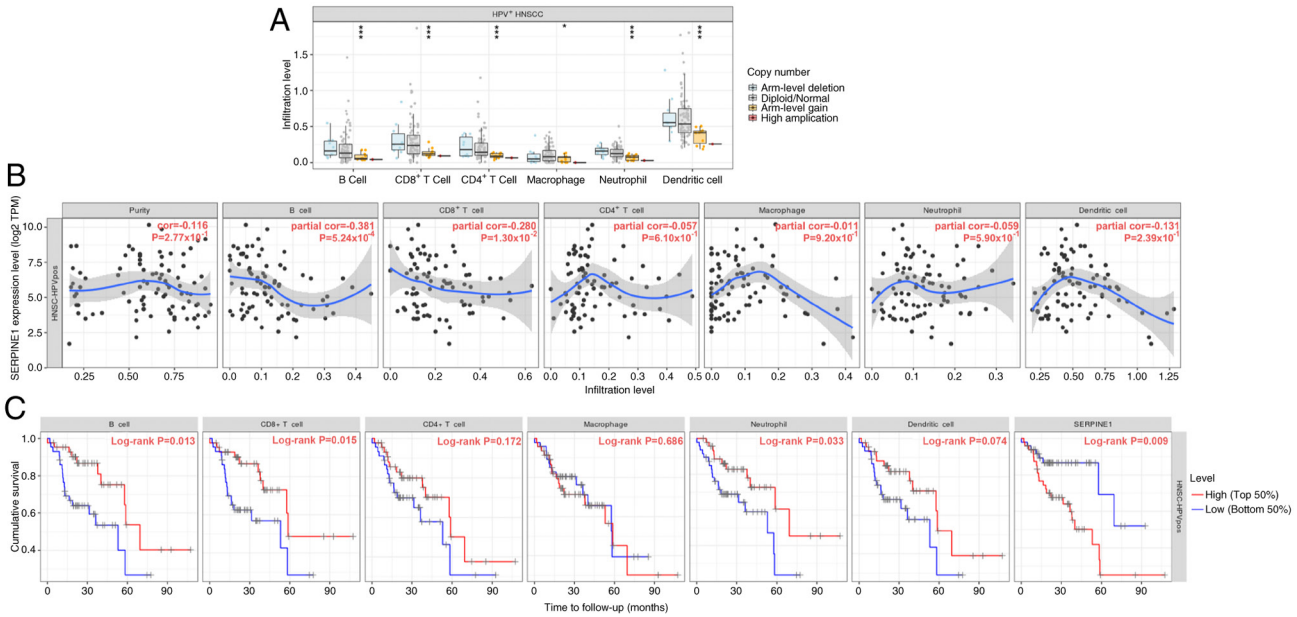

Figure 7. SERPINE1 shapes the tumor immune microenvironment of HPV⁺ HNSCC. (A) Through arm-level gain, the SCNA of SERPINE1 can significantly influence immune cell infiltrations. (B) Weak negative correlations between SERPINE1 expression level and infiltrations of B cells and CD8⁺ T cells in HPV⁺ HNSCC. (C) Prognostic value of B-cell and CD8⁺ T-cell infiltrations, along with SERPINE1 expression, for improved overall survival. *P<0.05 and ***P<0.001. SCNA, somatic copy number alteration; SERPINE1, serpin family member 1; HPV, human papillomavirus; HNSCC, head and neck squamous cell carcinoma; TPM, transcripts per million.

associated with poor prognosis in HPV⁺ HNSCC. Molecular docking showed that quercetin binds strongly to SERPINE1 protein, with a binding energy of -7.1 kcal/mol. Subsequent experiments demonstrated a dose-dependent downregulation in the expression of SERPINE1 at both the mRNA and protein levels in response to quercetin. These findings indicated that

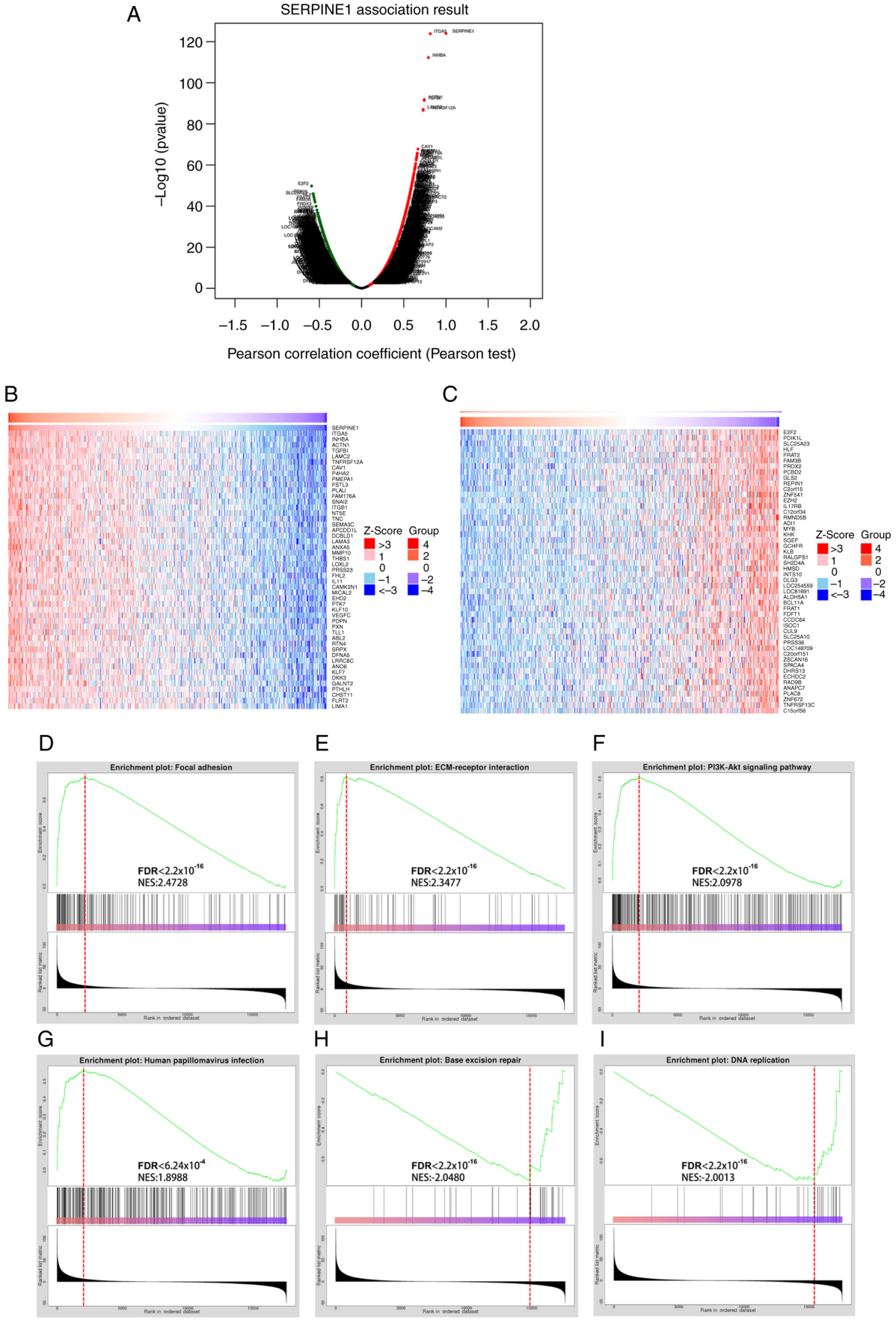

Figure 8. Co-expression analysis and GSEA of SERPINE1 in HNSCC. (A) Scatter plot representing the genes co-expressed with SERPINE1. (B) Top 50 genes positively associated with SERPINE1. (C) Top 50 genes negatively associated with SERPINE1. GSEA demonstrated a positive association between the SERPINE1 gene set and (D) focal adhesion.=, (E) ECM-receptor interaction, (F) PI3K-AKT signaling pathway and (G) human papillomavirus infection, a negative association between the SERPINE1 gene set and (H) base excision repair and (I) DNA replication. GSEA, Gene Set Enrichment Analysis; SERPINE1, serpin family member 1.

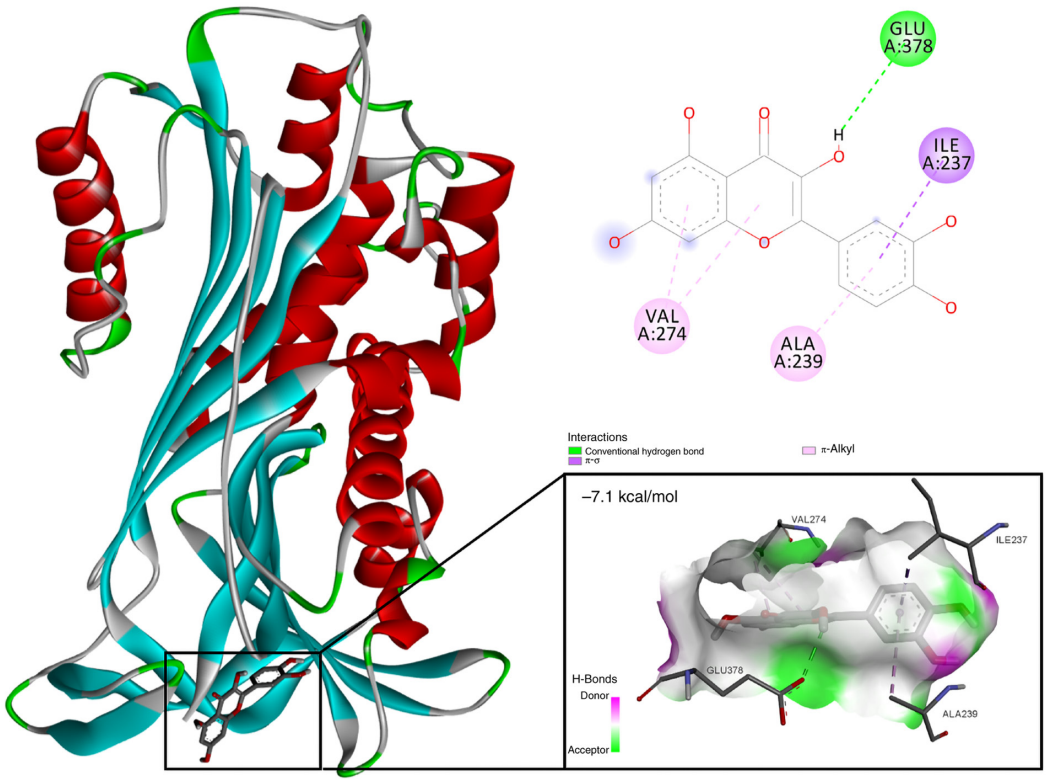

Figure 9. Molecular docking analysis. Molecular docking analysis of quercetin, a key component of *Aloe vera*, with the SERPINE1 protein revealed a binding energy of -7.1 kcal/mol. Quercetin establishes a hydrogen bond with GLU378, π-alkyl interactions with VAL274 and ALA239, and a π-σ interaction with ILE237, indicating strong binding affinity within the active site of SERPINE1. SERPINE1, serpin family member 1.

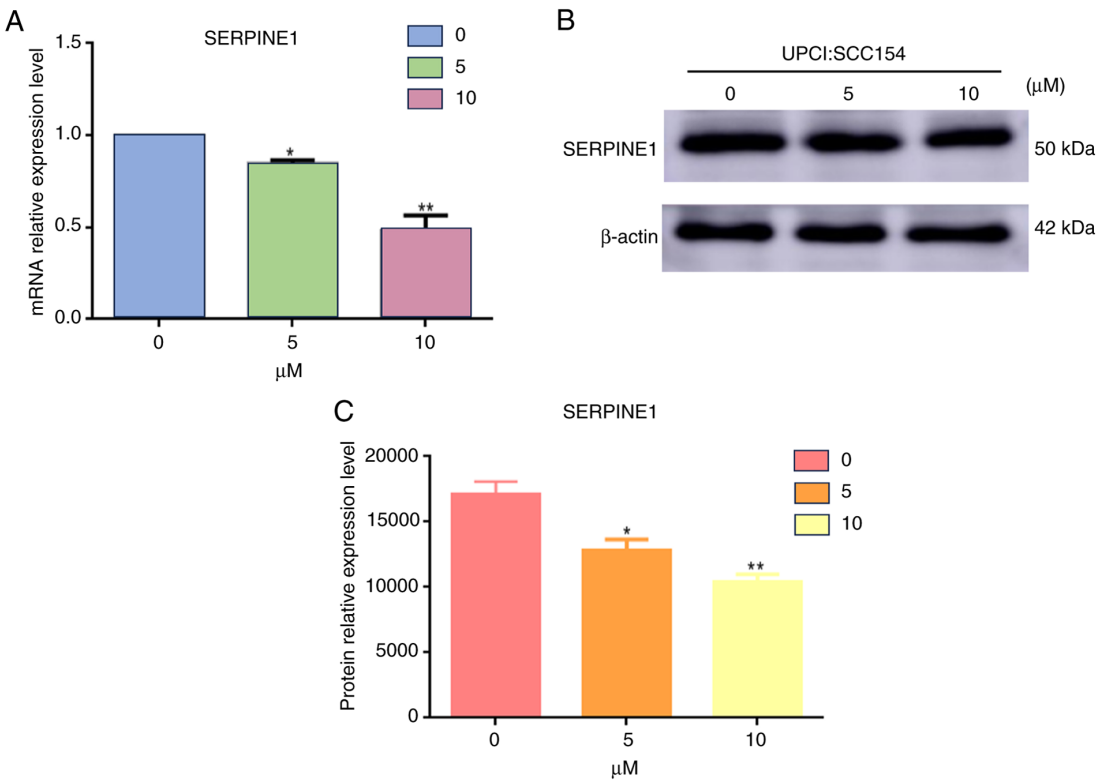

Figure 10. Effects of quercetin on SERPINE1 expression in HPV⁺ HNSCC cells. (A) Quercetin caused a dose-dependent decrease of SERPINE1 mRNA expression in HPV⁺ HNSCC cells, as measured after exposure to 0, 5 and 10 μM concentrations (*P<0.05; **P<0.01 vs 0 μM). (B) Western blot analysis further validated the downregulation of SERPINE1 protein expression following quercetin treatment. (C) Semi-quantitative bar graph showing the downregulation of SERPINE1 protein expression levels following *Aloe vera* treatment, as determined by densitometric analysis of western blotting data. Data are presented as the mean ± SEM (n=3) (*P<0.05; **P<0.01 vs. 0 μM). SERPINE1, serpin family member 1; HPV, human papillomavirus; HNSCC, head and neck squamous cell carcinoma.

quercetin may inhibit SERPINE1 by stabilizing its active conformation, potentially reversing the immunosuppressive TIME associated with elevated SERPINE1 expressions and SCNAs. Additionally, quercetin may exert anticancer effects by directly binding and inhibiting the HPV oncoprotein E6, further contributing to its potential as a multi-targeted anti-cancer agent in HPV+ HNSCC. Therefore, the present study presents a promising therapeutic strategy for ameliorating the TIME and enhancing treatment efficacy in HPV+ HNSCC, by targeting SERPINE1 with quercetin through a dual mechanism of both inhibiting its expression and stabilizing its conformation.

Although quercetin has been demonstrated to down-regulate SERPINE1 in HPV+ HNSCC cells, the current study only confirmed the results through the use of *in vitro* cell assays. Further investigations are needed to confirm its therapeutic efficacy as a bioactive ingredient and to reveal its related molecular mechanisms in complex tumor microenvironments *in vivo*. Additionally, natural products are distinguished by their multitarget and multi-pathway characteristics, implying their potential to elicit synergistic effects through diverse phytochemicals (39). The present study investigated the regulatory effects of quercetin targeting SERPINE1; however, further research is still needed to explore the potential synergistic effects among different active components and their combined regulatory impact on SERPINE1.

In summary, SERPINE1 is a highly promising biological marker and therapeutic target in HPV+ HNSCC. Quercetin has the potential to exert anticancer effects through targeting and inhibiting SERPINE1, and it may also capitalize on its antiviral properties. These findings indicate that quercetin, along with *Aloe vera*, could be compelling candidates for further investigation into the treatment of HPV+ HNSCC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KZ, YH and KW conceived and designed the present study. KZ, YH, YZ, XZ and SH collected the data. KZ, YH, YZ, XZ and SH analyzed and interpreted the data. KZ and YH wrote the manuscript. KW provided critical revisions important for the intellectual content. KZ and KW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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