Overexpression of STOML1 is associated with good prognosis in nasopharyngeal carcinoma

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Abstract. Nasopharyngeal carcinoma (NPC) is a prevalent head and neck malignancy characterized by high recurrence rate, adversely affecting patient prognosis. The present study aimed to identify key genes and mechanisms affecting NPC prognosis using DNA microarray, bioinformatics analysis and clinical data integration. The gene expression profile of patients with NPC with favorable (n=12) and unfavorable (n=8) prognoses was assessed using cDNA profiling. Bioinformatics analysis was performed to identify key prognostic factors, whilst immunohistochemistry assays were performed to evaluate the association between gene-protein expression in 107 NPC samples. The prognostic significance was assessed using Cox regression analysis, Kaplan-Meier curves, log-rank tests and receiver operating characteristic (ROC) analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to assess the underlying mechanisms. cDNA profiling identified six genes, including stomatin like 1 (STOML1), as significant prognostic factors. The Kaplan-Meier Plotter and The Cancer Genome Atlas database indicated that the mRNA expression levels of STOML1 were significantly associated (P<0.05) with head and neck squamous cell carcinoma prognosis. In addition, tissue microarray analysis revealed that high protein expression levels of STOML1 were significantly associated (P<0.05) with improved overall survival (OS) and disease-free survival (DFS). Furthermore, univariate and multivariate Cox analyses demonstrated that STOML1 expression is an independent prognostic factor for OS and DFS. ROC analysis also revealed improved predictive accuracy for 5-year OS when combining STOML1 expression with tumor-node-metastasis (TNM) staging [area under the curve (AUC)=0.874; P<0.001], compared with TNM staging alone (AUC=0.715; P=0.043) and STOML1 expression alone (AUC=0.774; P=0.010). Finally, GO and KEGG analyses demonstrated that the identified genes were mainly involved in pathways associated with apoptosis and cancer progression. Overall, the results of the present study suggested that STOML1 could serve a crucial role in NPC progression and could therefore serve as a valuable biomarker for NPC diagnosis and prognosis.

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most prevalent types of malignant tumors of the head and neck region; it ranks 23rd in global incidence but 21st in mortality, which is characterized by distinct geographic distribution and a strong tendency for metastasis (1). NPC incidence is particularly high in Southern China and parts of Southeast Asia (2). NPC is a multistage disease with a multifactorial etiology, involving gene-environment interactions, such as genetic susceptibility, exposure to chemical carcinogens and infection with the Epstein-Barr virus (EBV) (3,4). With the advancements in medical technology, the detection of early-stage NPC has steadily increased in recent years. However, the recurrence rate remains high (5,6). Currently, concurrent chemoradiotherapy and chemotherapy are the primary treatment approaches for NPC, with radiotherapy serving a pivotal role in disease management (7,8). However, despite improvements in staging and therapeutic approaches, the persistently high long-term recurrence rate (10-30%) still

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remains a major factor affecting the prognosis of patients with NPC (9). Therefore, identifying key genes associated with NPC prognosis and exploring their roles in patient outcomes are critical issues that need to be addressed.

Advances in DNA microarray technology have provided a valuable platform for identifying biomarkers that can enhance the assessment of tumor burden and cancer treatment efficacy (10-12). Paik *et al* (13) reported that a gene expression profile based on DNA microarray, when combined with multiple genes, could predict which patients with breast cancer could benefit from chemotherapy. Similarly, the gene signature used by Glinsky *et al* (14) revealed a high degree of prognostic accuracy for prostate cancer. In a study by Dave *et al* (15), biopsy samples from 191 untreated patients with follicular lymphoma were analyzed using DNA microarrays. The results revealed a marked association between survival prognosis and the molecular characteristics of non-malignant immune cells present in the tumor at diagnosis.

To the best of our knowledge, none of the available diagnostic methods have been sufficiently validated for clinical application, with the exception of EBV-DNA (16), which is closely associated with both the diagnosis and prognosis of patients with NPC. As a result, the current approaches focus on integrating microarray analysis with clinical data and bioinformatics to identify key genes that affect tumor prognosis and enhance diagnostic accuracy. This strategy aims to address the heterogeneity that complicates NPC risk stratification. Therefore, in the present study, DNA microarray analysis, integrated with bioinformatics and clinical data, was employed to identify key genes that could serve as prognostic markers in NPC.

Materials and methods

Tissue specimens. A total of 20 patients with differentiated squamous cell NPC were recruited prior to radiation therapy at the Institute of NPC, Affiliated with the People's Hospital of Guangxi Zhuang Autonomous Region (Nanning, China) between June 2003 and July 2004. Inclusion criteria were as follows: i) Histopathologically confirmed non-keratinizing squamous cell PC (WHO Type II); ii) age ≥18 years at diagnosis; iii) no prior history of radiotherapy, chemotherapy or surgical intervention for NPC; and iv) signed informed consent for tissue sample collection and follow-up procedures. Exclusion criteria were as follows: i) Presence of distant metastasis at initial diagnosis; ii) coexistence of other primary or metastatic malignancies; iii) severe comorbidities that may compromise treatment compliance or follow-up; iv) active autoimmune diseases requiring systemic immunosuppressive therapy; and v) loss to follow-up (incomplete clinical data or inability to assess treatment outcomes). These patients included 15 men (75%) and 5 women (25%), with a median age of 41.5 years (35.8, 51.0). Pre-treatment primary nasopharyngeal tumor tissues were obtained from patients with NPC through diagnostic biopsy and immediately stored at -80˚C until required for analysis. Written informed consent was obtained from all patients prior to biopsy procedures. The present study was approved by the Institutional Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region (approval no. KY-KJT-2024-43).

Patients were categorized into 'favorable' and 'unfavorable' prognosis groups, based on survival times (>3 or <3 years). The relevant patient characteristics are listed in Table I.

Isolation of RNA and cDNA synthesis. Total RNA was isolated from the tissue samples of 20 patients with NPC using a TRIzol™ reagent (Invitrogen™; Thermo Fisher Scientific, Inc.), as per the manufacturer's guidelines. The concentration and purity of each RNA sample were assessed using agarose gel electrophoresis, whilst RNA integrity was ensured by measuring the 260/280 nm absorbance ratio. Subsequently, total RNA was reverse-transcribed into cDNA using Oligo (dT) primers and the Superscript™ III RNase H-Reverse Transcriptase Kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The synthesized cDNA was stored at -20˚C for future use.

Microarray construction and probe preparation. The construction of the microarray using the tissues from 20 patients with NPC and probe preparation were performed as previously described (17).

Hybridization. The probe was dissolved in 20 µl hybridization buffer (5X SSC, 0.75 M NaCl, 0.075 M sodium citrate, 0.4% SDS and 50% formamide). The microarrays were pre-treated in a hybridization buffer supplemented with 0.5 mg/ml denatured salmon sperm DNA at 42˚C for 6 h. Subsequently, the fluorescent probe mixtures were denatured following heating at 95˚C for 5 min and were then applied to the pre-hybridized microarray under a cover slip. Hybridization was performed at 42˚C for 15-17 h. Post-hybridization, the chips were washed at 60˚C for 10 min in the following sequential solutions: 2X SSC with 0.2% SDS, followed by 0.1X SSC with 0.2% SDS and then in 0.1X SSC. Finally, the chips were air-dried at room temperature.

Microarray data analysis. Tissue specimens from 20 NPC patients with varying prognoses were used to construct cDNA microarrays (Biostar H-141s) (Shanghai Boxing Gene Chip, Co., Ltd.) following Brown's protocol, encompassing 9,646 genes. After normalizing ≥14 microarray datasets, detectable signals enabled evaluation of gene expression profiles across NPC patients with divergent prognoses. Differential gene expression analysis was performed using the limma package (version 3.54.1) (https://bioconductor.org/packages/limma/) in R software (version 4.2.1) (https://www.r-project.org/). To capture the emissions from Cy5 and Cy3, the microarrays were scanned using the ScanArray 4000 (GSI Lumonics, Inc.) at wavelengths of 635 and 532 nm, respectively. GenePix Pro 3.0 software (Molecular Devices, LLC) was utilized to process the captured images. The intensities for each spot at these two wavelengths corresponded to the quantities of Cy3-dUTP and Cy5-dUTP. The Cy5 to Cy3 ratios were calculated using the GenePix Pro 3.0 median ratio method. Data normalization was performed utilizing the default normalization factor provided by GenePix. Spots flagged as 'bad' or 'not found' by the software were excluded from the analysis. Only genes with raw intensity values of >200 counts for both Cy3 and Cy5 on each array were further analyzed.

Table I. Clinicopathological characteristics of patients with nasopharyngeal carcinoma.

Sample no.	Sex	Age, years	TNM stage	Clinical cancer stage	Overall survival, years	Prognosis
8	Male	65	T3N1M0	III	≥3	Favorable
19	Male	60	T2N2M0	III	≥3	Favorable
83	Male	54	T4N1M0	IVA	≥3	Favorable
93	Male	38	T3N0M0	III	≥3	Favorable
102	Male	42	T1N1M0	II	≥3	Favorable
108	Female	28	T3N2M0	III	≥3	Favorable
109	Female	50	T1N1M0	II	≥3	Favorable
122	Male	67	T3N3M0	IVA	≥3	Favorable
130	Male	60	T2N2M0	III	≥3	Favorable
157	Male	53	T4N0M0	IVA	≥3	Favorable
158	Male	32	T1N1M0	II	≥3	Favorable
170	Male	50	T2N3M0	IVA	≥3	Favorable
6	Male	34	T2N2M0	III	<3	Unfavorable
10	Male	51	T3N0M0	III	<3	Unfavorable
22	Male	55	T3N1M0	III	<3	Unfavorable
25	Male	41	T4N2M0	IVA	<3	Unfavorable
56	Male	51	T2N1M1	IVB	<3	Unfavorable
60	Male	45	T2N2M0	III	<3	Unfavorable
84	Male	51	T2N3M0	IVA	<3	Unfavorable
87	Male	41	T3N2M0	III	<3	Unfavorable

T, tumor; N, node; M, metastasis.

Immunohistochemistry. To assess the expression levels of stomatin like (STOML)1, ras-related protein 25 (RAB25), brain acid soluble protein 1 (BASP1), RAD50-interacting protein 1 (RINT1) and U2 snRNP associated SURP domain containing (U2SURP), a tissue microarray containing 107 primary NPC samples was purchased from Shanghai Outdo Biotech Co. Ltd. (cat. no. HNasN110Su01). Out of the 107 patients, there were 77 men (71.96%) and 30 women (28.04%), with a median age was 47.0 years (41.0-56.0). Ethical approval was obtained from the Ethics Committee of Shanghai Outdo Biotech Company (Shanghai, China; approval no. YBM-05-01).

Immunohistochemistry was performed on the 107 paraffin-embedded NPC tissue sections (4-μm thickness). Tissues were fixed in 10% neutral buffered formalin at room temperature for 24 h prior to paraffin embedding, as previously described (17). Briefly, antigen retrieval was performed by microwaving sections in 10 mM citrate buffer (pH 6.0) at 95-100˚C for 5 min. Prior to this step, tissue sections underwent deparaffinization and rehydration through a descending ethanol series (100% down to 70%), followed by endogenous peroxidase blocking. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol for 10 min at room temperature. To prevent non-specific binding, slides were incubated with 10% FBS/PBS for 30 min at room temperature. The sections were then incubated with antibodies against STOML1 (1:100; cat. no. HPA042353; Sigma-Aldrich; Merck KGaA), RAB25 (1:100; cat. no. Ml086507; Shanghai Enzyme-linked Biotechnology Co., Ltd.), BASP1 (1:200; cat. no. HPA045218; Sigma-Aldrich; Merck KGaA), RINT1 (1:100; cat. no. HPA019875; Sigma-Aldrich; Merck KGaA) and U2SURP (1:500; cat. no. HPA037546; Sigma-Aldrich; Merck KGaA) at 4˚C overnight. After incubation, the sections were washed three times with PBS (pH 7.4) and incubated in HRP-conjugated anti-rabbit (cat. no. A0239; Beyotime Institute of Biotechnology) secondary antibodies for 1 h at room temperature, followed by staining with DAB according to the GTVision™ II Detection System/Mo&Rb Kit (cat. no. GK500705; Shanghai GenTech Co., Ltd.) instructions. After DAB staining, sections were counterstained with Mayer's Hematoxylin Solution (cat. no. G1004; Wuhan Servicebio Technology Co., Ltd.) for 30 sec at room temperature, followed by differentiation in 1% hydrochloric acid-alcohol for 5 sec and bluing in saturated lithium carbonate solution for 30 sec. The sections were then dehydrated through an ascending ethanol series (70, 95 and 100%) and cleared in xylene, the sections were mounted and visualized under a light microscope (Leica DM2500; Leica Microsystems, Inc.).

Bioinformatics analysis. Gene expression comparisons between head and neck squamous cell carcinoma (HNSC) and normal tissues were performed using mRNA data from the TCGA-HNSC project (https://portal.gdc.cancer.gov/projects/TCGA-HNSC), Survival prognosis was evaluated through Kaplan-Meier analysis using the Kaplan-Meier Plotter database (https://kmplot.com/analysis). Additionally, the Tumor Immune Estimation Resource (TIMER) 2.0 database

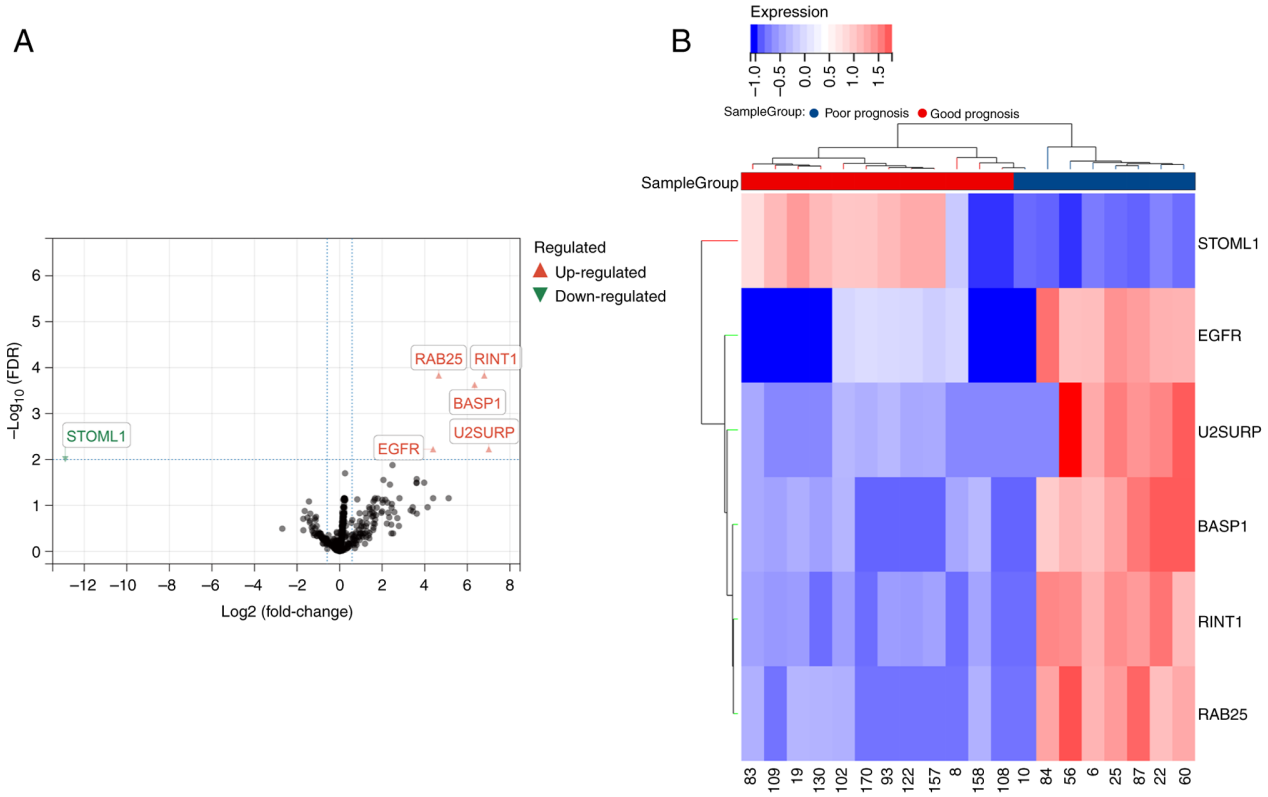

Figure 1. Differential gene expression analysis in patients with nasopharyngeal carcinoma with varying prognoses. (A) Volcano plot. (B) Heatmap. FDR, false discovery rate; STOML1, stomatin like 1; EGFR, epidermal growth factor receptor; RAB25, Ras-related protein 25; BASP1, brain acid soluble protein 1; RINT1, RAD50-interacting protein 1; U2SURP, U2 snRNP associated SURP domain containing.

(http://timer.cistrome.org) was employed to predict the correlations between gene mRNA expression levels.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analysis. GO and KEGG pathway analyses were performed using the clusterProfiler package (version 4.4.4) (https://bioconductor.org/packages/clusterProfiler/) in R software (version 4.2.1) (https://www.r-project.org/) with data from the microarray of tissues from 20 patients with NPC. ID conversion for the molecular input lists was facilitated using the org.Hs.eg.db package from the ID conversion library (https://bioconductor.org/packages/org.Hs.eg.db/). The enrichment results were visualized with ggplot2 (version 3.3.6) (https://cran.r-project.org/package=ggplot2), along with igraph (version 1.4.1) (https://cran.r-project.org/package=igraph) and ggraph (version 2.1.0) for enhanced graphical representation (https://cran.r-project.org/package=ggraph).

Predictive performance of receiver operating characteristic (ROC) curves in patients with NPC. ROC curve analysis, performed using data from the microarray of 107 primary NPC samples in GraphPad Prism 9 software (Dotmatics), was employed to evaluate the predictive performance of different clinical variables, such as TNM stage and protein expression levels, in NPC prognosis.

Statistical analysis. Statistical analyses were performed using SPSS software (v22.0; IBM Corps.) and GraphPad Prism v.9.0. (Dotmatics). Data are expressed as the mean ± standard deviation. Unpaired student's t-test was applied to evaluate significant differences between two groups. Kaplan-Meier survival curves were plotted to assess the association between gene expression and overall survival (OS) and disease-free survival (DFS) in patients with NPC. Prognostic cut-off values for BASP1 and STOML1 expression were determined using X-tile software v3.6.1 (http://www.tissuearray.org/rimmlab/Xtile.htm). The significance was then determined using the log-rank test. Cox regression analyses were performed to identify prognostic factors that could affect OS. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of differentially expressed genes. The study cohort included 12 patients with favorable outcomes and eight with unfavorable ones. Differentially expressed genes were identified using the following criteria: $|Log_2$ fold change$|>1.5$, $P<0.05$ and false discovery rate <0.01. Data analysis was performed using the Limma package in R. A total of six genes were identified to be associated with NPC prognosis. Among them, epidermal growth factor receptor (EGFR), RINT1, BASP1, U2SURP and RAB25 were upregulated in patients with unfavorable outcomes, whilst STOML1 was downregulated in this group (Fig. 1). As EGFR is a well-characterized prognostic marker in several types of cancer, it was excluded from further analysis (18).

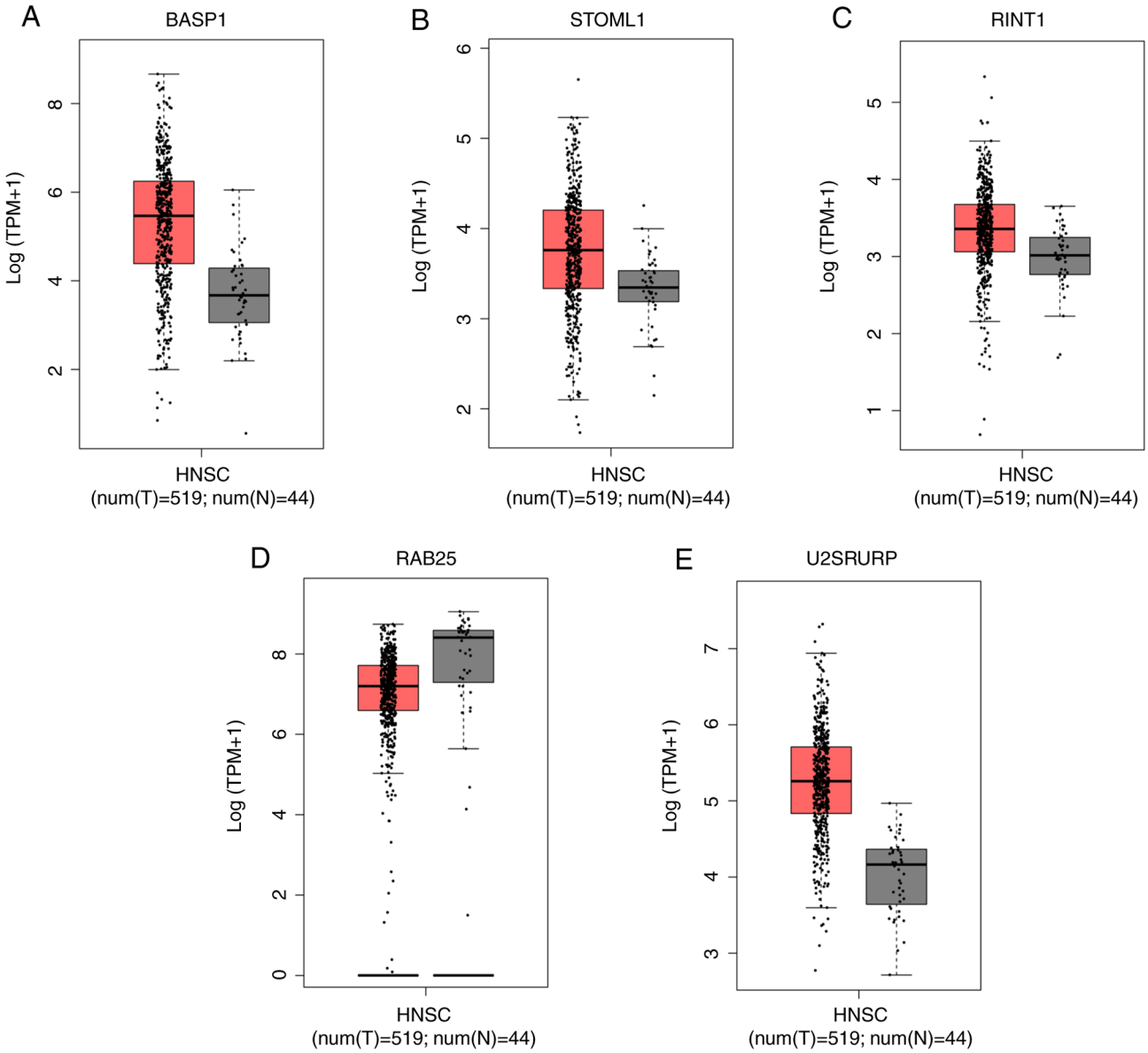

Figure 2. Differential mRNA expression of differentially expressed genes between HNSC and normal tissues, based on The Cancer Genome Atlas database analysis. (A) BASP1; (B) STOML1; (C) RINT1; (D) RAB25; and (E) U2SURP. HNSC, head and neck squamous cell carcinoma; BASP1, brain acid soluble protein 1; STOML1, stomatin like 1; RINT1, RAD50-interacting protein 1; RAB25, Ras-related protein 25; U2SURP, U2 snRNP associated SURP domain containing; T, tumor tissue; N, normal tissue.

Identification of key genes. The TCGA database was used to assess the differential expression of five genes in HNSC tissues compared with normal ones, and the results revealed that the mRNA expression levels of RINT1, BASP1, U2SURP and STOML1 were increased in HNSC tissues, whereas RAB25 expression was decreased (Fig. 2). Additionally, the Kaplan-Meier plotter online database was utilized to assess the associations between the mRNA expression levels of the aforementioned five genes in HNSC tumor tissues and OS. The results revealed that patients with high BASP1 mRNA expression levels had significantly shorter OS rates compared with those with low BASP1 expression. By contrast, elevated STOML1 mRNA expression was significantly associated with markedly longer OS compared with low STOML1 expression ($P<0.05$; Fig. 3).

Gene survival analyses. To further assess the reliability of DNA microarray technology and database-predicted results, an immunohistochemistry analysis was performed on tissue samples from 107 patients with NPC (Figs. S1-5). Due to their aforementioned OS results, the analysis focused on assessing the expression levels of STOML1 and BASP1. Additionally, patients were categorized into high and low expression groups for both proteins using the X-tile tool (19), which provided optimal cut-off values. Kaplan-Meier survival curves were utilized to evaluate patient survival, whilst the log-rank test was applied to assess the effect of BASP1 and STOML1 expression on OS and DFS in patients with NPC. The results demonstrated that high STOML1 expression was significantly associated with both improved OS and DFS rates compared with low STOML1 expression ($P<0.05$). By contrast, high BASP1 expression was significantly associated with worse

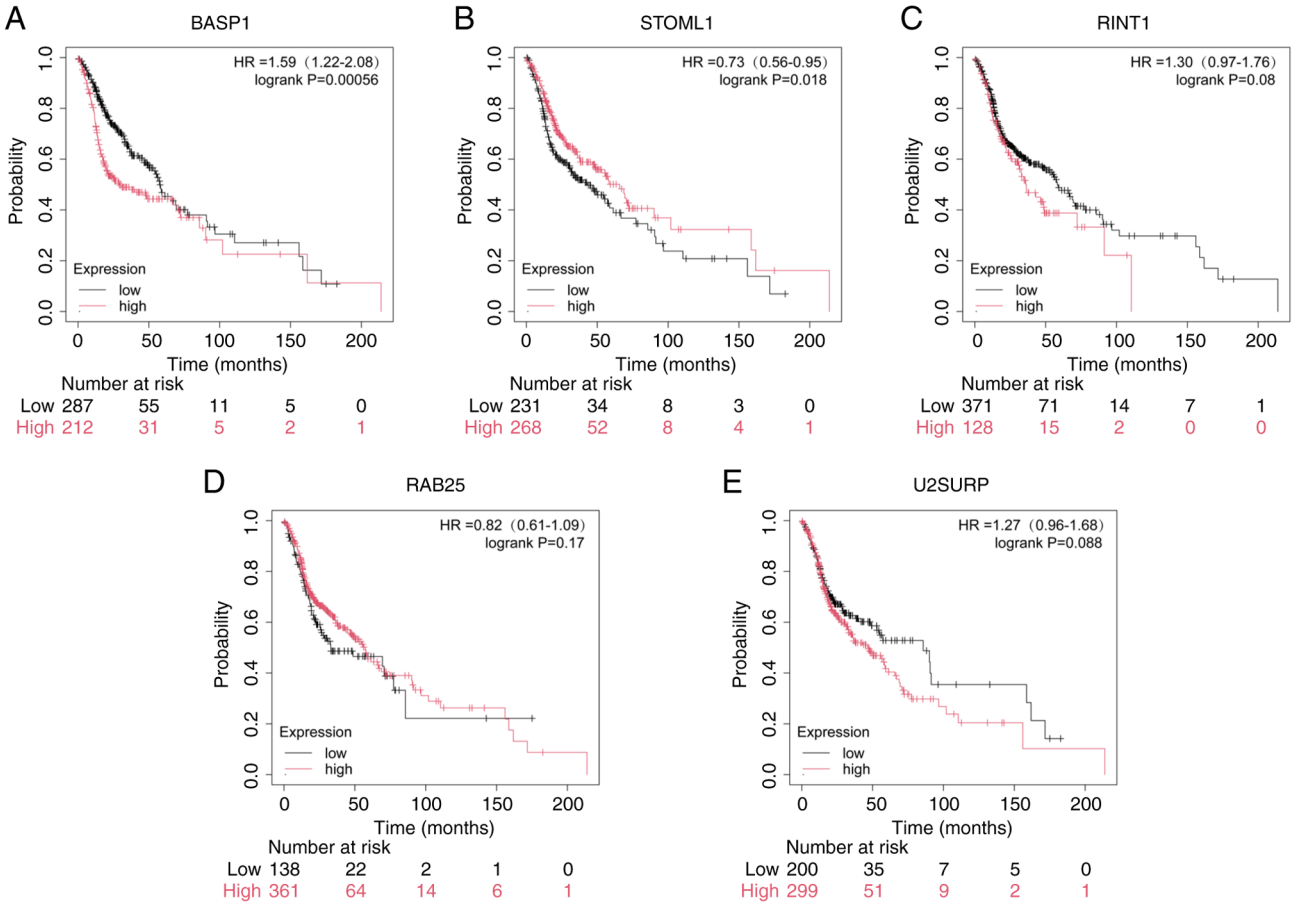

Figure 3. Association between mRNA expression levels of differentially expressed genes and overall survival in patients with head and neck squamous cell carcinoma, based on the Kaplan-Meier plotter database analysis. (A) BASP1; (B) STOML1; (C) RINT1; (D) RAB25; and (E) U2SURP. BASP1, brain acid soluble protein 1; STOML1, stomatin like 1; RINT1, RAD50-interacting protein 1; RAB25, Ras-related protein 25; U2SURP, U2 snRNP associated SURP domain containing; HR, hazard ratio.

DFS rates compared with low BASP1 expression, but not OS (P<0.05; Fig. 4). Furthermore, Cox univariate and multivariate analyses were performed to assess the association between protein expression, clinical characteristics, OS and DFS. The analyses revealed that STOML1 expression was an independent prognostic factor for both OS (Table II) and DFS (Table III) in patients with NPC. These preliminary findings suggest that STOML1 could serve a crucial role in the development and progression of NPC.

Predictive performance of TNM stage and STOML1 in the prognosis of patients with NPC. ROC curve analysis was performed to assess the predictive performance of different models in 5-year OS in 107 patients with NPC. More specifically, the predictive significance of TNM staging, the current gold standard for NPC prognosis (20), the protein expression levels of STOML1 and that of a combined model incorporating both factors were evaluated. The results revealed an AUC value of 0.774 [95% confidence interval (CI), 0.580-0.968; P=0.010] for STOML1, 0.715 (95% CI, 0.556-0.875; P=0.043) for TNM staging and 0.874 (95% CI, 0.766-0.982; P<0.001) for the combined model (Fig. 5). These findings indicate that the protein expression levels of STOML1, whether analyzed independently or in combination with TNM staging, demonstrate improved prognostic accuracy compared with TNM staging alone for predicting the outcomes of patients with NPC.

GO and KEGG analyses. GO and KEGG pathway enrichment analyses were performed on the differentially expressed genes identified through the DNA microarray analysis of tissues from 20 patients with NPC to assess the potential molecular mechanisms underlying the effect of STOML1 on NPC prognosis. GO functional enrichment analysis revealed that NPC prognosis was associated with several biological processes, including 'inactivation of epidermal growth factor', 'K63-linked polyubiquitination-dependent protein binding' and 'negative regulation of the TLR4 signaling pathway'. In addition, KEGG pathway enrichment analysis demonstrated that NPC prognosis may be closely associated with several pathways, such as the 'PI3K-Akt signaling pathway', 'EBV infection' and 'microRNAs in cancer' (Fig. 6). Furthermore, an analysis using the TIMER 2.0 database revealed a significant positive correlation between STOML1 and the mRNA expression levels of BAX, FAS and caspase-9, which are key genes involved in the apoptosis pathway (21) (Fig. 7). The aforementioned results suggest that STOML1 could affect the apoptosis-related signaling pathways, thus potentially suppressing NPC progression.

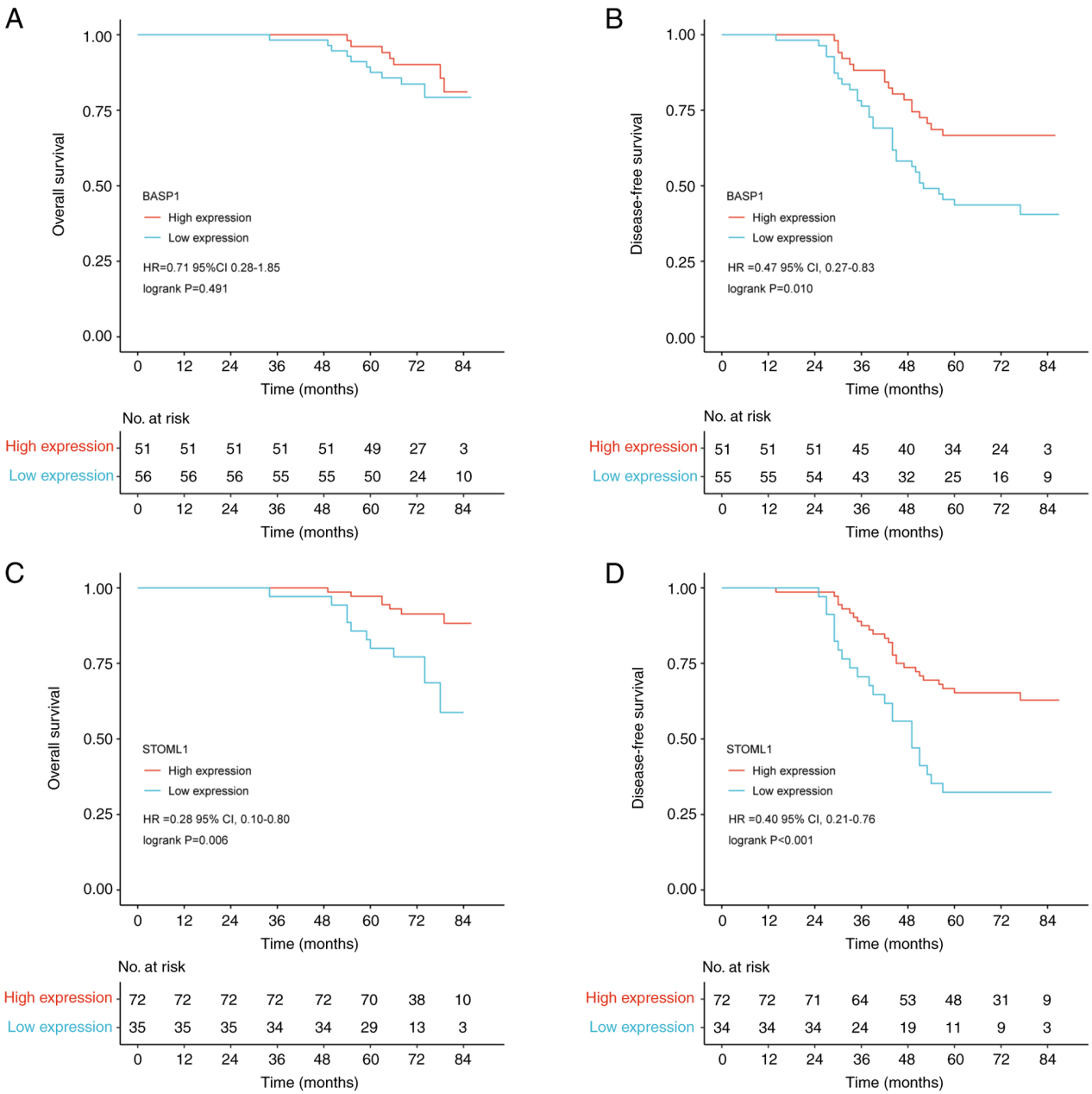

Figure 4. Association between BASP1 and STOML1 mRNA expression levels with OS and DFS in patients with nasopharyngeal carcinoma. (A) BASP1 and OS; (B) BASP1 and DFS; (C) STOML1 and OS; and (D) STOML1 and DFS. BASP1, brain acid soluble protein 1; STOML1, stomatin like 1; RINT1, RAD50-interacting protein 1; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

Discussion

NPC remains one of the most prevalent types of cancer in China (22). However, due to the absence of early symptoms, patients are commonly diagnosed at advanced stages, therefore leading to a poor prognosis (23,24). Currently, the TNM staging system and World Health Organization histological grading are commonly employed to predict survival and prognosis in patients with NPC (25,26). Nonetheless, clinical outcomes can notably vary among patients with the same stage and similar treatment regimens, thus indicating that the molecular mechanisms driving the disease are highly complex (27,28).

Our previous research has focused on identifying biomarkers that could affect the prognosis and radiotherapy sensitivity of patients with NPC using microarray technology and bioinformatics analysis. Yang *et al* (29) identified key genes, such as zinc finger protein 608 and colony stimulating factor 1 receptor (CSF1R), utilizing the DNA microarray technology. These genes which may serve notable roles in NPC prognosis following radiotherapy and hold promise as biomarkers for predicting radiotherapy outcomes. Furthermore, Chen *et al* (30) demonstrated that CSF1R promoted the proliferation, migration and invasion of NPC cells via activating the PI3K/Akt signaling pathway, whilst simultaneously inhibiting cell apoptosis, eventually affecting patient prognosis. However, further data mining combined with clinical data is essential to identify candidate biomarkers and elucidate their mechanisms of action, thus improving prognosis and potentially aiding the early diagnosis of NPC.

Table II. Univariate and multivariate Cox analysis of stomatin like 1 expression for the overall survival of patients with nasopharyngeal carcinoma.

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Sex (male vs. female)	1.225	0.413-3.813	0.689			
Age (continuous)	0.986	0.946-1.028	0.505			
Tumor size (continuous)	0.860	0.382-1.936	0.716			
T stage (T3 + T4 vs. T1 + T2)	15.829	3.633-68.965	<0.001	4.860	0.640-36.935	0.127
N stage (N1 + N2 + N3 vs. N0)	7.382	0.982-55.488	0.052			
TNM stage (III + IV vs. I + II)	21.430	2.850-161.117	0.003	4.752	0.296-76.381	0.271
STOML1 expression (high vs. low)	0.276	0.104-0.730	0.010	0.302	0.114-0.797	0.016

HR, hazard ratio; CI, confidence interval; T, tumor; N, node; M, metastasis; STOML, stomatin like 1.

Table III. Univariate and multivariate Cox analysis of stomatin like 1 expression for the disease-free survival of patients with nasopharyngeal carcinoma.

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Sex (male vs. female)	1.813	0.881-3.732	0.106			
Age (continuous)	1.003	0.978-1.028	0.836			
Tumor size (continuous)	1.203	0.791-1.830	0.388			
T stage (T3 + T4 vs. T1 + T2)	2.455	1.402-4.299	0.002	0.835	0.362-1.925	0.673
N stage (N1 + N2 + N3 vs. N0)	1.819	0.909-3.638	0.091			
TNM stage (III + IV vs. I + II)	3.173	1.729-5.823	<0.001	3.251	1.307-8.089	0.011
STOML1 expression (high vs. low)	0.398	0.227-0.700	0.001	0.467	0.264-0.827	0.009

HR, hazard ratio; CI, confidence interval; T, tumor; N, node; M, metastasis; STOML1, stomatin like 1.

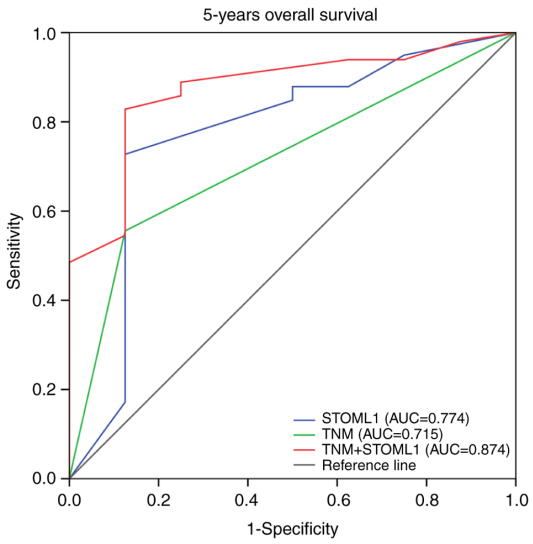

Figure 5. Time-dependent receiver operating characteristic curves of STOML1 protein expression, TNM staging system and their combination for evaluating the specificity and sensitivity in predicting 5-year overall survival in 107 patients with nasopharyngeal carcinoma. STOML1, stomatin like 1; TNM, tumor-node-metastasis; AUC, area under the curve.

In the current study, gene expression profiling was performed using DNA microarray technology was used on cancerous tissues from 12 patients with NPC, including four with a favorable prognosis and eight with a poor prognosis. The analysis revealed that STOML1 was significantly differentially expressed in NPC tissue and closely associated with the prognosis of NPC. Furthermore, tissue microarray analysis identified the protein expression levels of STOML1 as an independent prognostic factor for both OS and DFS in patients with NPC. Notably, STOML1 displayed superior predictive accuracy for patient prognosis compared with TNM staging, whether used alone or in combination with the TNM classification. Moreover, whilst the present study highlights the potential of STOML1 as a prognostic biomarker, further validation is required to assess its clinical utility with established biomarkers such as EBV DNA.

STOML1 is primarily localized in cellular vesicles and membranes. The STOML1 protein contains unique domains at both its N- and C-termini, thus allowing its binding with other regulatory factors and involvement in the regulation of proto-oncogene degradation (31-33). Other members of the same family, such as STOML2 and STOML3, have been implicated in the development of several types of cancers. Zheng *et al* (34)

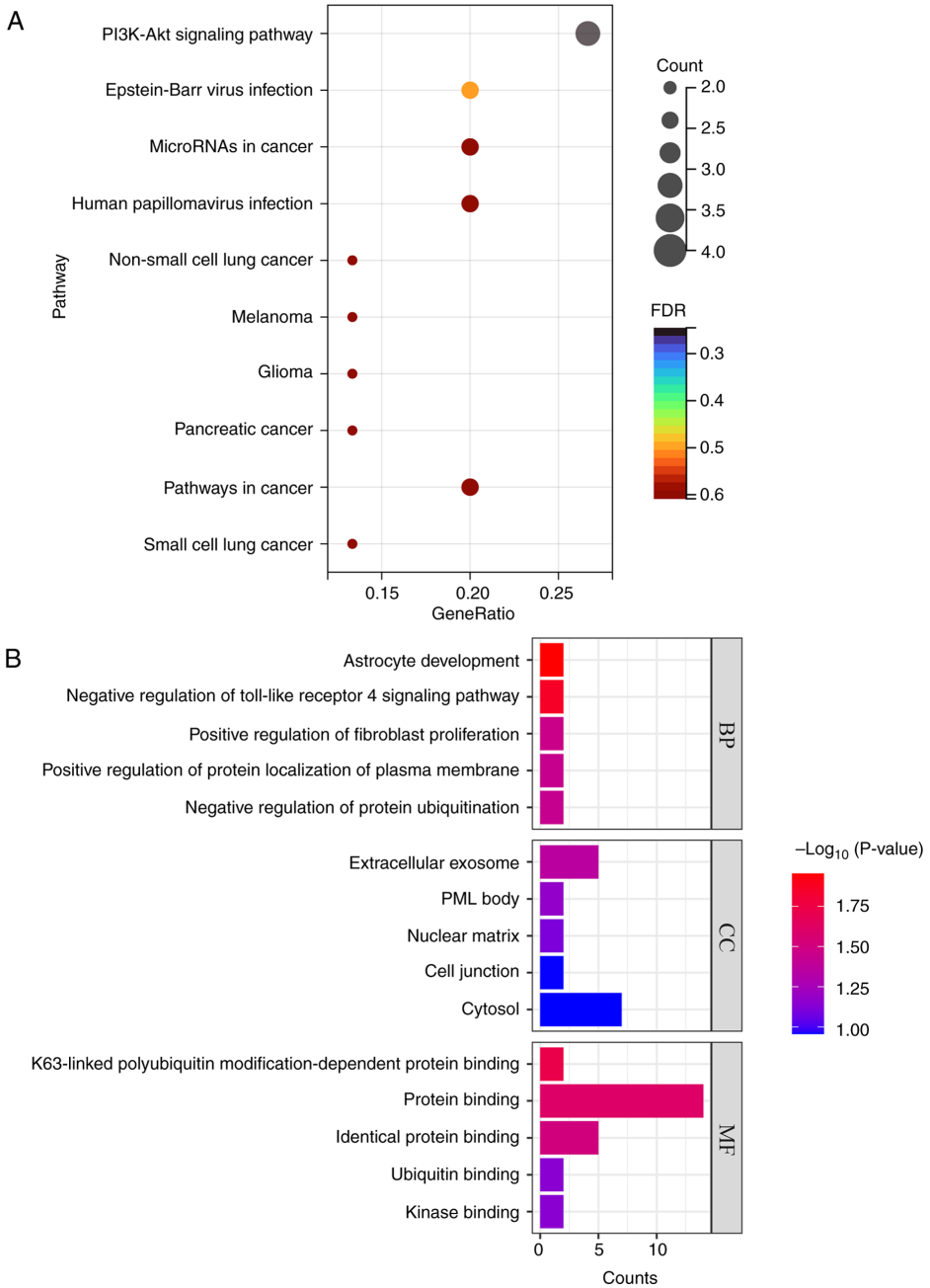

Figure 6. Analysis of the enriched pathways and functional characterization. (A) Pathway enrichment and (B) Gene Ontology functional analysis of differentially expressed genes associated with NPC prognosis in 20 patients with NPC. FDR, false discovery rate. NPC, nasopharyngeal carcinoma.

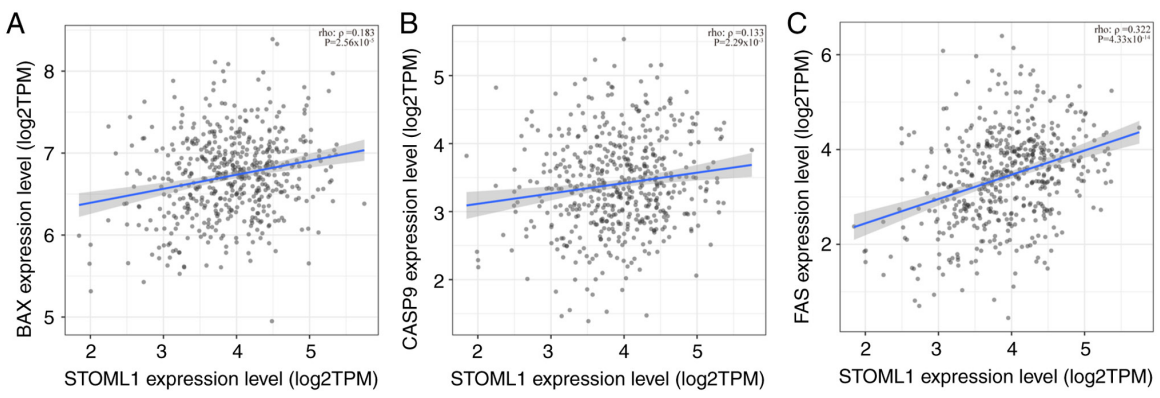

Figure 7. Correlation analysis of mRNA expression between STOML1 and key apoptosis-related genes in head and neck squamous cell carcinoma, based on the Tumor Immune Estimation Resource 2.0 database. (A) BAX; (B) FAS; and (C) CASP9. STOML1, stomatin like 1; CASP9, caspase-9; TPM, transcripts per million.

reported that STOML2 could promote the proliferation and metastasis of hepatocellular carcinoma cells via inducing mitophagy through PTEN induced kinase 1 regulation. Additionally, a previous study reported that STOML3 was highly expressed in mesenchymal gliomas (35). The oncogenic role of STOML1 has been supported by several studies. For example, a gene signature involving STOML1 was reported to predict survival outcomes in patients with breast cancer (36). Furthermore, in 2020, a study reported that both STOML1 and STOML2 were highly expressed in oral squamous cell carcinoma and were notably associated with patient prognosis (37). However, the expression profile and role of STOML1 in NPC have not been previously investigated, to the best of our knowledge.

The results of the present study suggest that STOML1 expression could serve as an indicator of prognosis for NPC. Furthermore, analysis using the TIMER 2.0 database revealed a significant positive association between the mRNA expression levels of STOML1 and those of BAX, FAS and caspase-9, three critical apoptosis-related genes. These findings aligned with the results of the KEGG pathway and GO enrichment analyses, indicating that STOML1 could regulate and mediate apoptosis, thereby affecting the prognosis of patients with NPC.

However, the present study has several limitations that warrant further discussion. Firstly, the sample size was relatively small and the follow-up period was insufficient, thus possibly resulting in negative results, such as for TNM staging in the multivariate analysis. Therefore, the results of the present study need to be further assessed using multi-center randomized controlled studies with larger sample sizes. Secondly, the current study was restricted to bioinformatics analysis and clinical specimens. Therefore, the results should be further verified through *in vivo* or *in vitro* experiments or via exploring the underlying molecular mechanisms. Moreover, although the findings of the present study are promising, further research is needed to elucidate the precise mechanisms contributing to the poor prognosis of NPC.

Overall, STOML1 expression may be a valuable biomarker for the diagnosis and prognosis of NPC. However, further investigation is needed to uncover the underlying molecular mechanisms and clinical application of STOML1.

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Availability of data and materials

The raw sequencing data generated in the present study are not publicly available due to restrictions imposed by the Institutional Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region, in compliance with national regulations governing the use and sharing of human genomic data, but may be requested from the corresponding author. All other data generated in the present study may be requested from the corresponding author.

Authors' contributions

HTL, ML and JXC contributed to the design and conceptualization of the study. SHQ, JJC and PL performed tissue sampling and data collection from patients. JLL and YQL performed the bioinformatics analysis. HTL drafted the initial manuscript, whilst SD and JZG wrote the methods/results sections, with data interpretation, and ensured the manuscript met formatting/ethical standards. ZG and WLH performed bioinformatic analysis of gene expression profiling data. HTL and JXC confirm the authenticity of all the raw data. All authors accept responsibility for every aspect of the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region approved the present study (approval no. KY-KJT-2024-43), which was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient and study samples were pseudonymized.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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