# Silencing of small nuclear ribonucleoprotein polypeptide B inhibits the progression of esophageal squamous cell carcinoma

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**Abstract.** Esophageal squamous cell carcinoma (ESCC) is a highly aggressive malignancy with poor prognosis. Small nuclear ribonucleoprotein polypeptide B (SNRPB) has been implicated in the progression of various types of cancer; however, the specific role of this protein in ESCC remains unclear. The present study aimed to investigate the expression and functional significance of SNRPB in ESCC. Bioinformatics analysis was performed to assess SNRPB expression and its clinical relevance in ESCC. Immunohistochemical staining of ESCC tissue samples demonstrated significantly elevated SNRPB expression in tumor tissues, compared with adjacent non-cancerous esophageal tissues. Analysis of public database (TCGA) and institutional clinical data revealed that high SNRPB expression was associated with poor prognosis and advanced clinical stage (T stage), respectively. To evaluate the functional role of SNRPB, a lentivirus-based short hairpin RNA vector was used to inhibit endogenous SNRPB expression in ESCC cell lines. Functional assays, including colony formation, flow cytometry and western blot analysis, demonstrated that SNRPB knockdown significantly inhibited cell proliferation, induced cell cycle arrest and promoted apoptosis. Moreover, molecular analysis indicated that SNRPB knockdown led to modulation of the cell cycle and proteins associated with apoptosis. These findings suggested that SNRPB is a key regulator of ESCC progression, through impacting cell proliferation and apoptosis. Collectively, results of the present study demonstrated that SNRPB may act as a potential prognostic biomarker and therapeutic target for ESCC.

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## Introduction

Esophageal cancer is among the leading causes of cancer-related mortality worldwide (1). Each year, ~16,120 individuals in the United States are diagnosed with esophageal cancer (2). Despite advances in diagnostic techniques and treatment options, the prognosis for individuals with esophageal cancer remains unfavorable, with 5-year survival rates typically ranging between 15 and 25% (3,4). Notably, esophageal squamous cell carcinoma (ESCC) accounts for ~90% of cases of esophageal cancer (5). However, despite advances in screening and therapeutic interventions, patient outcomes remain poor, and a considerable subset of patients continues to experience disease recurrence or progression (6,7). Therefore, the discovery of novel targets for the management of ESCC are required.

In the search for such novel targets, dysregulation of fundamental cellular processes such as alternative splicing has emerged as a key area of investigation in oncology. Alternative splicing is a pivotal regulatory mechanism that governs gene expression and increases protein diversity (8). This process is carried out by the spliceosome, a complex ribonucleoprotein machine. Small nuclear ribonucleoprotein polypeptides B (SNRPB) and B' are essential components of this machinery (9).

Accumulating evidence has associated SNRPB to various human diseases. For instance, aberrations in SNRPB contribute to the pathogenesis of cerebrocostomandibular syndrome (10,11) and carry out a role in regulating bone and cartilage differentiation (12). In the context of cancer, SNRPB has been identified as a key oncogenic driver. It has been shown to promote cell proliferation in lung cancer (13) and regulate the cell cycle to drive the progression of liver cancer (14). Furthermore, in cervical and thyroid cancer, SNRPB exerts its effects by inhibiting the tumor suppressor protein p53 (15,16). Although previous studies have demonstrated that SNRPB plays a tumor-promoting role in various types of cancer, including effects on cell proliferation and apoptosis (13,17-19), there is currently a lack of data regarding its expression, functional role and clinical relevance in ESCC.

Therefore, the present study focused on elucidating the role of SNRPB in ESCC.

The present study aimed to characterize SNRPB expression in ESCC and clarify its functional role in tumor progression; SNRPB expression and clinical relevance in ESCC was first analyzed using bioinformatics tools and immunohistochemical staining of clinical tissue samples, subsequently the biological effects of SNRPB silencing on ESCC cells were investigated using lentivirus-mediated shRNA knockdown *in vitro*. Thus, we hypothesized that SNRPB may promote ESCC progression through impacting cell proliferation and apoptosis, positioning it as a potential prognostic biomarker and therapeutic target.

## Materials and methods

*Prognostic analysis of SNRPB mRNA*. In the present study, Gene Expression Profiling Interactive Analysis (GEPIA; (http://gepia.cancer-pku.cn/)) was used to assess the differential expression of SNRPB between ESCC and matched healthy tissues (20). Transcriptome data and clinical information of 161 patients with esophageal cancer and 12 healthy esophageal tissue samples were downloaded from The Cancer Genome Atlas (TCGA; https://www.cancer.gov/ccg/research/genome-sequencing/tcga). Data were obtained from The Cancer Genome Atlas esophageal carcinoma (ESCA) cohort, which includes both esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). The SNRPB mRNA expression matrix was extracted from the dataset for subsequent analysis. The association between the expression levels of SNRPB and the survival outcomes of patients with ESCC was assessed using Kaplan-Meier survival analysis. Univariate and multivariate Cox regression analyses were performed via the 'survival' R software package (version 3.8-3) (21) to explore the independent prognostic factors of patients with ESCC (21).

*Clinical sample collection*. Samples of both neoplastic and pair-matched adjacent non-cancerous tissues, preserved in paraffin blocks, were collected from a cohort of 50 patients diagnosed with ESCC at the Affiliated Hospital of North Sichuan Medical College, Sichuan, China. The adjacent tissues were obtained from sites at least 5 cm away from the tumor margin and were histologically confirmed to be free of cancer cells by a certified pathologist. These patients received surgical interventions at the Affiliated Hospital of North Sichuan Medical College (Sichuan, China) from January 2019 to December 2021. The present study was approved by the Medical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, Sichuan, China (approval no. 2023ER344-1) and followed the guidelines outlined in the Declaration of Helsinki. As the present study was retrospective and patient data was anonymized, the Medical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College waived the requirement for informed consent. In total, 50 patients with ESCC (median age, 60 years; range, 45-77; 38 men and 12 women) were included, with tumor stages I (n=15), II (n=9), III (n=22) and IV (n=4). Characteristics of the 50 patients are summarized in Table SI.

Inclusion criteria were as follows: i) Histologically confirmed primary ESCC; ii) a history of curative resection between January 2019 and December 2021; and iii) no history of neoadjuvant radiotherapy or chemotherapy prior to surgery. Patients were excluded from the present study according to the following criteria: i) Incomplete clinical or follow-up data; and ii) a history of other synchronous or metachronous malignant tumors.

*Immunohistochemical analysis*. Immunohistochemistry was performed to assess the expression of SNRPB in tissues. Tissue samples were formalin-fixed and paraffin-embedded, with 4-$\mu$m-thick serial sections prepared for staining. Permeabilization was not required for the staining procedure. Prior to antibody incubation, sections were blocked with 5% bovine serum albumin in phosphate-buffered saline (PBS) at room temperature for 1 h. The primary antibody against SNRPB (cat. no. 10278-1-AP; Proteintech Group, Inc.) was diluted at 1:100 and incubated with the sections at 4˚C overnight (12 h). The secondary antibody used was HRP-conjugated goat anti-rabbit IgG (cat. no. SA00001-2; Proteintech Group, Inc.), diluted at 1:500 and incubated at room temperature for 1 h. All stained sections were observed and imaged using a Leica DM4 B upright light microscope (Leica Microsystems). To ensure the specificity and reliability of the staining, positive and negative controls were included in each staining batch. Colon cancer tissue sections, known to have high expression of SNRPB, were used as a positive control. For the negative control, the primary antibody was replaced with PBS. The immunoreactive score (IRS) scoring system was used to evaluate the expression of SNRPB (22).

*Cell culture and transfection*. ESCC cell lines, KYSE150, KYSE510, KYSE30 and KYSE410, were purchased from Saiku Biotechnology. All cell lines were cultivated in RPMI-1640 medium enriched with 10% fetal bovine serum, and maintained in an incubator at 37˚C with 5% $CO_2$. After confirming the high expression of SNRPB in ESCC tissues (Fig. 1), western blot analysis was performed to detect its expression in ESCC cell lines, which showed markedly higher endogenous SNRPB levels in KYSE30 and KYSE510 cells compared to the other tested lines (Fig. 2A). These two cell lines were thus selected for subsequent functional knockdown experiments. ShRNA constructs were integrated into the hU6-MCS-CBh-gcGFP-IRES-puromycin vector, and lentiviral particles with a titer of $1x10^8$ TU/ml were used for transduction; 2 $\mu$l of lentiviral particles were added per well in a 6-well plate, combined with HiTransG A Lentiviral Transduction Reagent (GeneChem, Inc.). The transfection was performed at 37˚C with 5% $CO_2$ for 8 h, and all subsequent functional assays were conducted at a 48 h time interval post-transfection. The lentiviral infection was performed at a multiplicity of infection of 10, which was determined as optimal in preliminary experiments to achieve high transduction efficiency with minimal cytotoxicity. Design and production of lentiviral constructs were carried out by Shanghai GeneChem Co., Ltd. All procedures involving lentiviral particles were performed in a Biosafety Level 2 (BSL-2) containment facility. The specific shRNA sequences used for SNRPB knockdown were as follows: shSNRPB#1, CCACAAGGAAGAGGTACTGTT; shSNRPB#2, CCTCCCAAAGATACTGGTATT; and shSNRPB#3, GCAGCATATTGATTACAGGAT. The sequence for the negative control (NC) was as follows: shNC, TTCTCCGAACGTGTCACGT.

*Colony formation assay*. Following transfection with shSNRPB#1, KYSE 30 and KYSE 510 cells were maintained in RPMI-1640 medium with 10% fetal bovine serum at 37˚C with 5% $CO_2$ for 12 days post-transfection, with the medium replaced every three days and no additional drug treatment applied in this experiment. Subsequently, cells were digested to prepare single-cell suspensions, and 1,000 cells per well were inoculated in 6-well plates, with control cells treated with a blank vector. After ~10 days, colonies were fixed with 4% paraformaldehyde at room temperature for 15 min, then stained with 0.1% crystal violet solution at room temperature for 20 min. Colonies containing >50 cells were counted to calculate the clonogenic survival rate. Colony counting was performed manually by two independent investigators who were blinded to the experimental group assignments.

*Cell cycle analysis*. SNRPB-knockdown KYSE30 and KYSE510 cells (transfected with shSNRPB#1) and negative control cells (transfected with shNC) were used for this assay, and analysis was performed 48 h after lentiviral transfection (consistent with the time interval for other subsequent functional experiments). For sample preparation, the cells were harvested by trypsinization with 0.25% trypsin-EDTA (Gibco; Thermo Fisher Scientific, Inc.), washed twice with pre-chilled PBS, and fixed with 70% cold ethanol at 4˚C for 24 h for cell cycle arrest. A Cell Cycle Detection kit (Nanjing KeyGen Biotech Co., Ltd.) was then used to assess cell cycle distribution according to the manufacturer's protocol. Analysis was conducted via a NovoCyte flow cytometer (NovoCyte 3130; ACEA Biosciences, Inc.), which allowed precise cell cycle phase determination.

*Apoptosis analysis*. SNRPB-knockdown KYSE30 and KYSE510 cells (transfected with shSNRPB#1) and negative control cells (transfected with shNC) were used for this assay. For apoptosis analysis, the Annexin V-APC/PI Apoptosis Detection kit (cat. no. KGA1030-100; Nanjing KeyGen Biotech Co., Ltd.) was utilized according to the manufacturer's protocol. A NovoCyte flow cytometer (ACEA Biosciences, Inc.) was used to quantify the proportion of apoptotic cells.

*Western blot analysis*. Intracellular proteins were obtained by lysing SNRPB-knockdown and negative control KYSE30 and KYSE510 cells using RIPA buffer (Beijing Applygen Technologies Inc.), with the addition of phosphatase inhibitors. Total proteins were quantified using a BCA Protein Assay Kit (Beyotime Biotechnology) to determine the concentration, and 30 $\mu$g of total protein was loaded into each lane for separation by SDS-PAGE on a 12% gel and transferred onto a PVDF membrane (Sigma-Aldrich; Merck KGaA). Membranes were subsequently blocked with a solution containing 5% skimmed milk powder (Beyotime Biotechnology) for 2 h at room temperature. Following blocking, membranes were incubated with the following primary antibodies overnight at 4˚C: Anti-SNRPB (cat. no. FNab08070; 1:1,000; FineTest), anti-GAPDH (cat. no. EM1101; 1:50,000; HUABIO), anti-CDK1 (cat. no. ET1607-54; 1:1,000; HUABIO), anti-CCNA2 (cat. no. : ET1612-26; 1:1,000; HUABIO), anti-BAX (cat. no. ET1603-34; 1:5,000; HUABIO) and anti-Bcl-2 (cat. no. : HA721235; 1:2,000; HUABIO). Following primary incubation, membranes were incubated with a secondary antibody (cat. no. FNSA-0004; 1:5,000; FineTest, goat anti-rabbit IgG HRP-conjugated) for 1 h at room temperature. Protein bands were visualized using a ChemiDoc™ XRS+ system (Universal Hood II; Bio-Rad Laboratories, Inc.). Protein expression was quantified using ImageJ software (version 1.54f; National Institutes of Health) with GAPDH serving as the loading control.

*Statistical analysis*. R software (version, 4.2.2; Foundation for Statistical Computing) and GraphPad Prism 8 (Dotmatics) were used for data analysis and visualization. Differences between two groups were evaluated using paired or unpaired Student's t tests for pairwise comparisons of independent or matched samples (specified in corresponding figure legends). One-way ANOVA followed by Dunnett's post hoc test was used for multiple group comparisons. The associations between SNRPB expression and clinicopathological characteristics were assessed using the chi-square ($\chi^2$) test for variables meeting the expected count assumption; Fisher's exact test (or the Freeman-Halton extension for >2x2 contingency tables) was used for Histological grade, Location and M stage (23). Cox regression analysis identified independent prognostic factors for esophageal cancer. $P<0.05$ was considered to indicate a statistically significant difference. All *in vitro* experiments were performed in triplicate, as independent biological replicates.

## Results

*SNRPB is highly expressed in ESCC tissues*. Online analysis using the GEPIA database confirmed the overexpression of SNRPB in esophageal cancer tissues compared with normal tissues (Fig. 1A). Survival analysis revealed a significant correlation between high SNRPB expression and reduced overall survival [hazard ratio (HR), 1.98; 95% confidence interval (CI), 1.17-3.34; P=0.009], as shown in Fig. 1B. Both univariate and multivariate analyses further demonstrated the prognostic value of SNRPB expression levels, along with tumor stage, in predicting patient outcomes ($P<0.05$ for both; Fig. 1C and D).

Immunohistochemical analysis further corroborated these findings, revealing elevated SNRPB protein expression levels in ESCC tissues (Fig. 1E). The IRS for SNRPB was significantly greater in tumor tissues when compared with that in adjacent non-cancerous tissues (P=0.012; Fig. 1F; paired Student's t test). Thus, results of the present study suggested that SNRPB expression may be associated with the clinicopathological features of patients with ESCC. A retrospective analysis of clinical data obtained from a cohort of 50 patients diagnosed with ESCC was conducted. The cohort comprised 38 male (76%) and 12 female (24%) patients, with a median age of 60 years (range, 45-77 years). Participants were divided into two groups, 'low' and 'high' expression, according to the median IRS for SNRPB obtained through immunohistochemical analysis. The full clinicopathological characteristics are detailed in Table I.

Specifically, individuals with an IRS ≤3.33 were placed in the low-expression category, and those individuals with an IRS >3.33 were placed in the high-expression category. The associations between SNRPB expression levels and a range of clinical characteristics were evaluated using a $\chi^2$ test. This analysis revealed a statistically significant correlation between high SNRPB protein expression and advanced T stage in patients with ESCC (P=0.025; Table I).

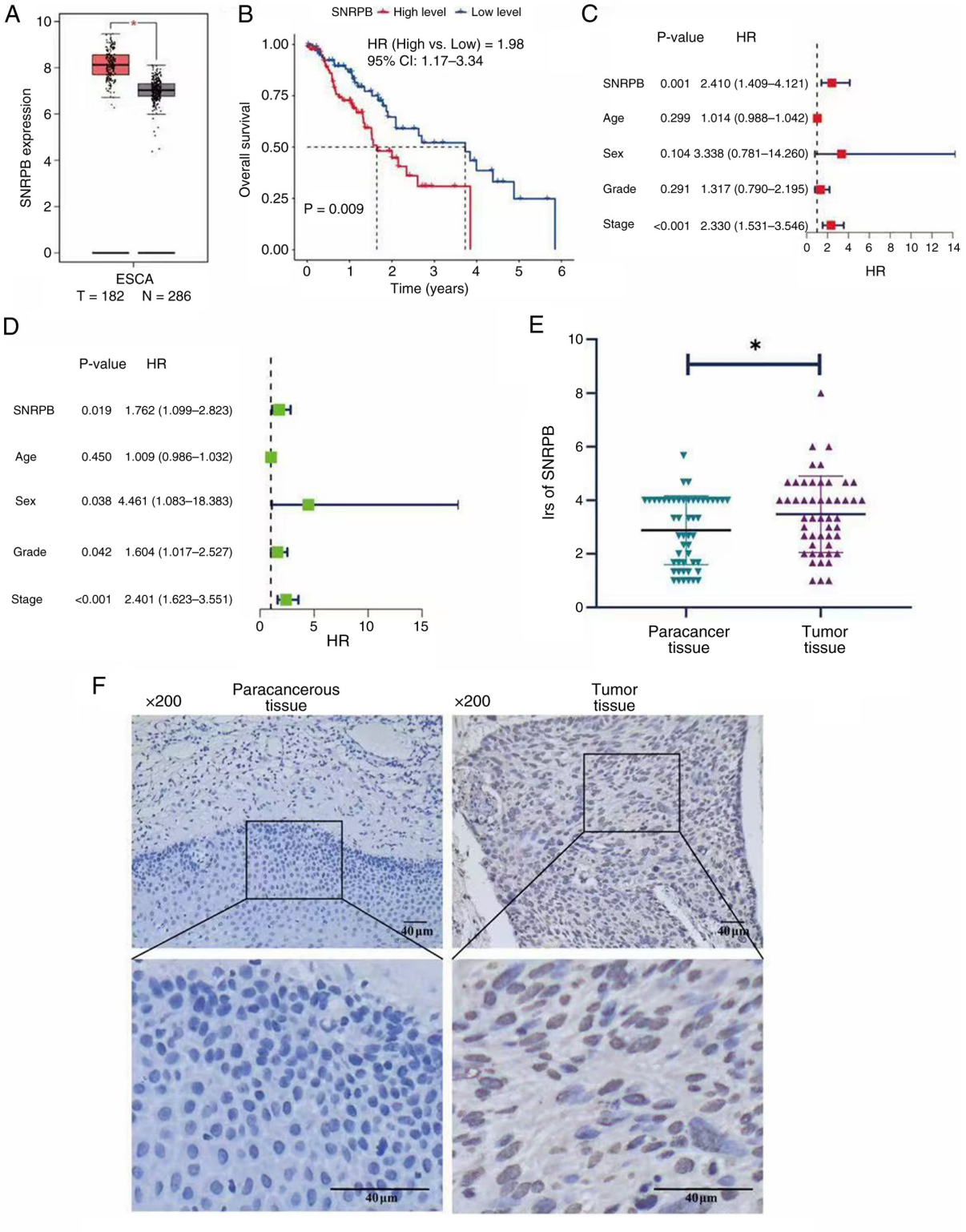


Figure 1. SNRPB is associated with poor prognosis in patients with ESCC and is highly expressed in ESCC tumor tissues. (A) Expression of SNRPB in the Gene Expression Profiling Interactive Analysis esophageal cancer and adjacent tissue datasets. (B) Kaplan-Meier survival analysis based on SNRPB expression levels. (C) Univariate analysis of SNRPB expression in the esophageal cancer dataset. (D) Multivariate analysis of SNRPB expression and clinical variables in the esophageal cancer dataset. (E) Comparative analysis of the IRS derived from the immunohistochemical staining of 50 ESCC tissues and corresponding paracancerous tissues. (F) Representative images of immunohistochemical staining of ESCC tissues and adjacent paracancerous tissues along with pathological magnification. *P<0.05; paired Student's t-test. HR, hazard ratio; SNRPB, small nuclear ribonucleoprotein polypeptide B; IRS, immunoreactive score; ESCC, esophageal squamous cell carcinoma; tumor tissue; N, normal tissue.

*Knockdown of SNRPB inhibits cell proliferation in ESCC.* To further investigate the impact of SNRPB on the biological behaviors of ESCC, previous research (13-15) has established SNRPB as a significant prognostic gene in multiple human malignancies. The expression levels of SNRPB were assessed in four ESCC cell lines, KYSE150, KYSE510, KYSE30 and

Table I. Correlations between SNRPB expression and the clinicopathological characteristics of patients with ESCC.

| Characteristics | No. of patients | Low SNRPB expression | High SNRPB expression | Test method/ statistic | P-value |
|---|---|---|---|---|---|
| Age | | | | $\chi^2$=0.045 | 0.832 |
| <60 | 18 | 9 | 9 | | |
| ≥60 | 32 | 17 | 15 | | |
| Sex | | | | $\chi^2$=1.937 | 0.164 |
| Male | 32 | 19 | 13 | | |
| Female | 18 | 7 | 11 | | |
| History of smoking | | | | $\chi^2$=0.855 | 0.355 |
| Yes | 20 | 12 | 8 | | |
| No | 30 | 14 | 16 | | |
| History of alcohol consumption | | | | $\chi^2$=0.142 | 0.706 |
| Yes | 18 | 10 | 8 | | |
| No | 32 | 16 | 16 | | |
| Histological grade | | | | F-H | 0.987 |
| G1 | 17 | 9 | 8 | | |
| G2 | 31 | 16 | 15 | | |
| G3 | 2 | 1 | 1 | | |
| Location | | | | F-H | 0.742 |
| Upper | 7 | 3 | 4 | | |
| Middle | 35 | 18 | 17 | | |
| Lower | 8 | 5 | 3 | | |
| TNM stage | | | | $\chi^2$=2.039 | 0.153 |
| IA + IB + IIA + IIB | 24 | 15 | 9 | | |
| IIIA + IIIB + IVA + IVB | 26 | 11 | 15 | | |
| T stage | | | | $\chi^2$=5.059 | 0.025[a] |
| T1 + T2 | 27 | 18 | 9 | | |
| T3 + T4 | 23 | 8 | 15 | | |
| N stage | | | | $\chi^2$=0.349 | 0.555 |
| N0 | 23 | 13 | 10 | | |
| N1 + N2 + N3 | 27 | 13 | 14 | | |
| M stage | | | | Fisher's exact | 0.225 |
| M0 | 48 | 26 | 22 | | |
| M1 | 2 | 0 | 2 | | |

[a]P<0.05 is considered to indicate a statistically significant difference. For variables with expected frequencies <5 in >20% of cells (Histological grade, Location, M stage), Fisher's exact test or Freeman-Halton extension to a 2x2 Fisher's test was used as appropriate. F-H, Freeman-Halton extension to a 2x2 Fisher's test.

KYSE410. Results of the western blot analysis revealed notably high SNRPB expression levels in KYSE30 and KYSE510 cell lines compared with KYSE150 and KYSE410 cells (Fig. 2A). Consequently, these two cell lines were selected for SNRPB knockdown. In total, three distinct shRNAs targeting SNRPB, shSNRPB#1, shSNRPB#2 and shSNRPB#3, were transfected into KYSE30 and KYSE510 cells. Moreover, western blot analysis was performed to determine knockdown efficiency, and the results demonstrated that shSNRPB#1 exhibited the highest level of knockdown efficiency compared with shSNRPB#2, shSNRPB#3 and shNC (Fig. 2B). Thus, shSNRPB1#1 shRNA was selected for use in subsequent experiments. As previous findings revealed that SNRPB expression was associated with tumor T stage (13,15,24,25), a colony formation assay was performed to assess the impact of SNRPB knockdown on the proliferation of cells. Based on three independent experiments, the results indicated that SNRPB knockdown markedly reduced colony formation capacity. Specifically, the number of colonies was significantly lower in the knockdown group compared with the shNC control group (Fig. 2C and D).

*SNRPB knockdown inhibits ESCC cell cycle progression.* To further elucidate the impact of SNRPB on the growth of

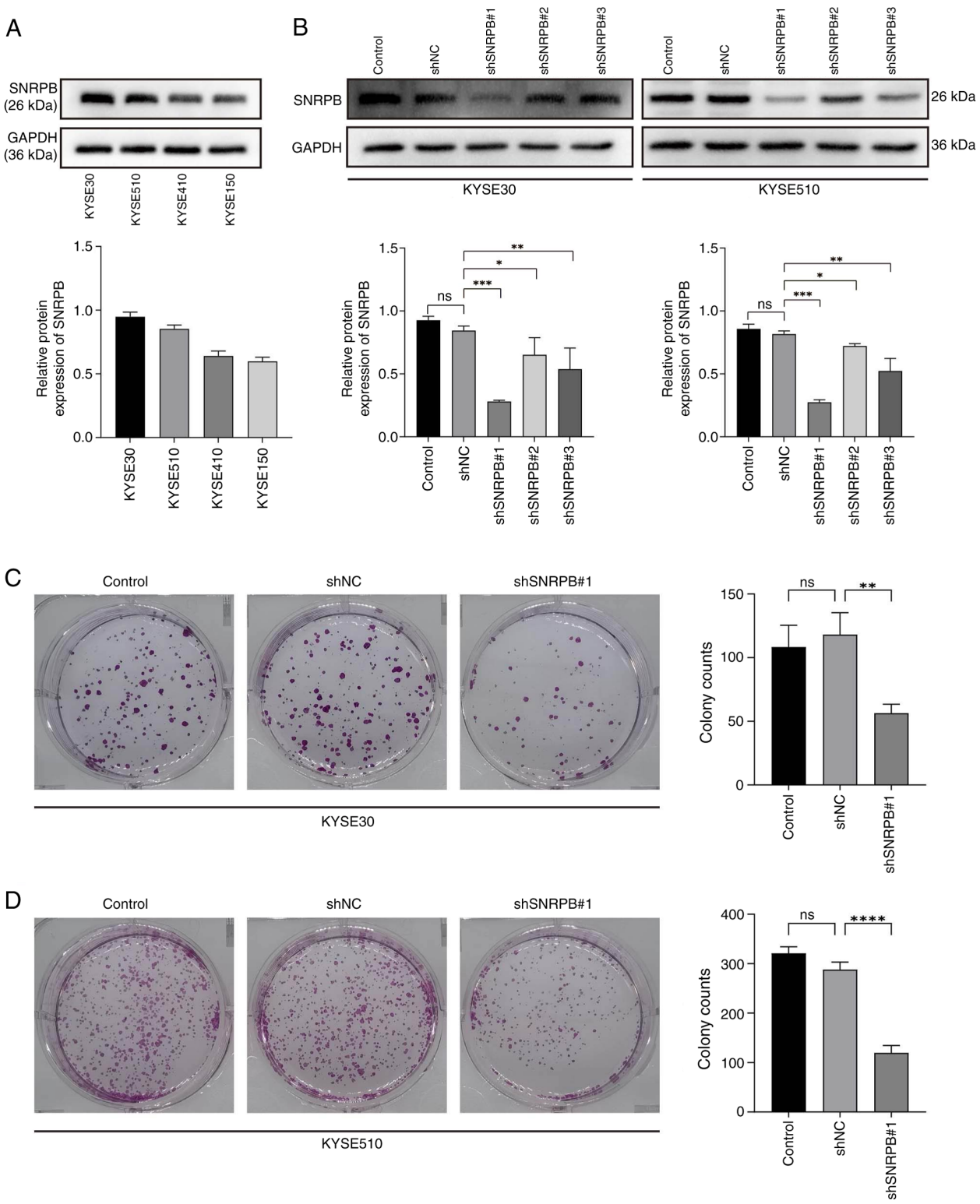


Figure 2. Silencing of SNRPB inhibits esophageal squamous cell carcinoma cell proliferation. (A) WB analysis was performed to determine the endogenous expression levels of SNRPB in four ESCC cell lines. (B) The knockdown efficiency of SNRPB shRNA in KYSE30 and KYSE510 cells was confirmed by WB. The effect of SNRPB knockdown on the proliferation of (C) KYSE30 and (D) KYSE510 cells was evaluated using a colony formation assay. Data are presented as the mean ± standard deviation from three independent experiments. Statistical significance was determined using an unpaired Student's t-test. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001, $^{****}$P<0.0001; one-way ANOVA followed by Dunnett's post hoc test. WB, western blotting; SNRPB, small nuclear ribonucleoprotein polypeptide B; shRNA, short hairpin RNA; ns, non-significant; NC, negative control.

ESCC cells, cell cycle analysis was conducted using KYSE30 and KYSE510 cell lines. Analysis suggested cell cycle arrest as flow cytometry analysis demonstrated that SNRPB knockdown increased the percentage of cells arrested in the G2/M phase from 12.3 to 28.7% in KYSE30 cells and from 10.5 to 25.2% in KYSE510 cells (Fig. 3A). To investigate the molecular mechanism underlying this arrest, western blot analysis was performed, which revealed that SNRPB

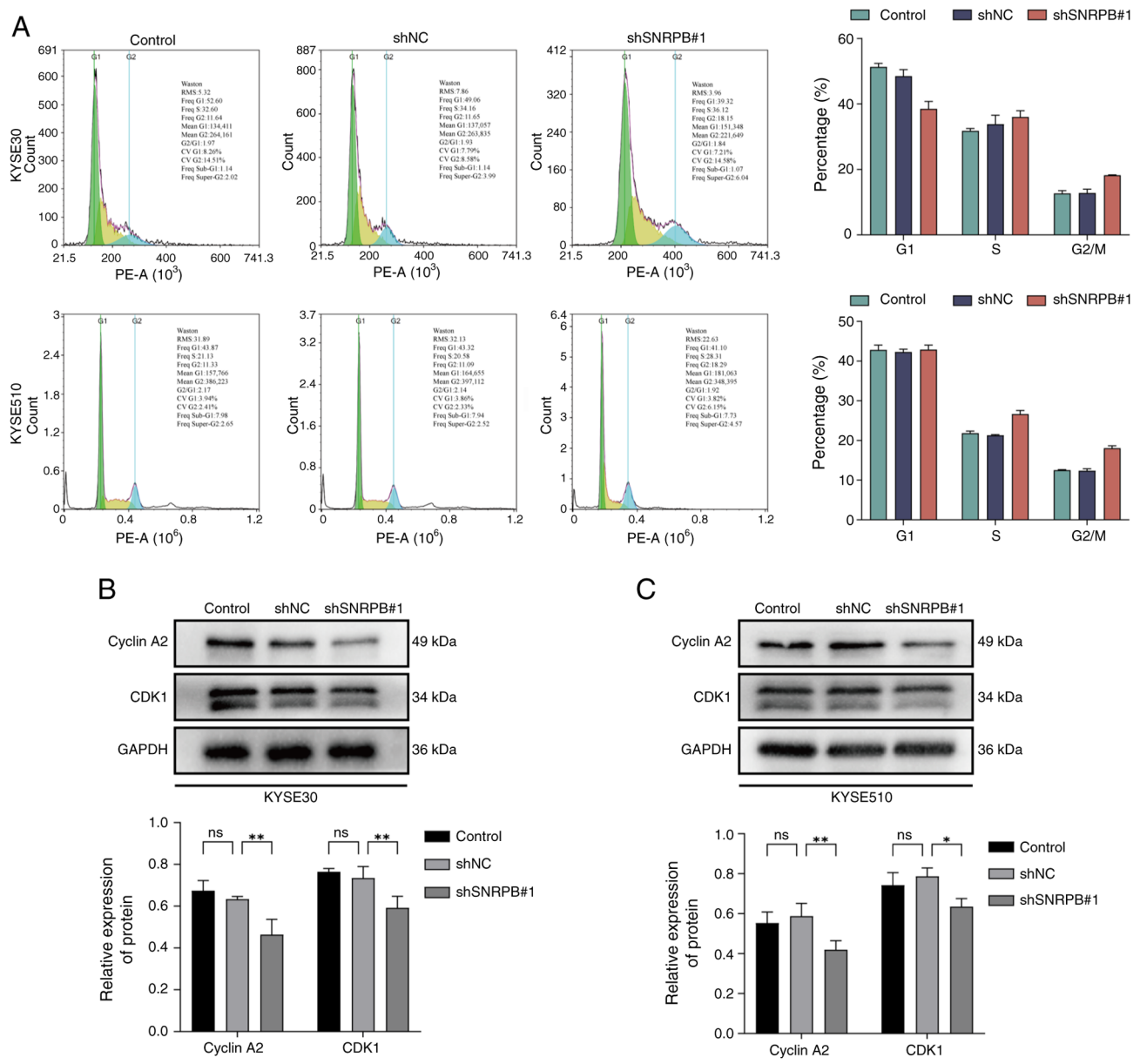


Figure 3. Silencing SNRPB arrests the cell cycle of esophageal squamous cell carcinoma cells. (A) Cell cycle distribution of the KYSE30 and KYSE510 cell lines after transduction with lentiviral shNC or SNRPB1#1 shRNA. Cell cycle analysis. SNRPB-knockdown KYSE30 and KYSE510 cells (transfected with shSNRPB#1) and negative control cells (transfected with shNC) were used for this assay; the analysis was performed 48 h after lentiviral transfection (the same time interval as subsequent functional experiments). For sample preparation, the cells were harvested by trypsinization with 0.25% trypsin-EDTA (Gibco; Thermo Fisher Scientific, Inc.), washed twice with pre-chilled phosphate-buffered saline (PBS), and fixed with 70% cold ethanol at 4˚C for 24 h for cell cycle arrest. After fixation, the cells were processed for cell cycle staining and detection according to the instructions of the Cell Cycle Detection kit (Nanjing KeyGen Biotech Co., Ltd.). Analysis was conducted via a NovoCyte flow cytometer (NovoCyte 3130; ACEA Biosciences, Inc.), which allowed precise cell cycle phase determination. The expression levels of cyclin proteins in (B) KYSE30 cells and (C) KYSE510 cells. Data are presented as the mean ± standard deviation. Statistical significance was determined using Student's t-test. $^{*}$P<0.05, $^{**}$P<0.01; one-way ANOVA followed by Dunnett's post hoc test. shRNA, short hairpin RNA; ns, non-significant; NC, negative control; SNRPB, small nuclear ribonucleoprotein polypeptide B.

knockdown led to significant reductions in the expression levels of Cyclin A2 and CDK1 in both KYSE30 and KYSE510 cell lines compared with the shNC group (KYSE30-CDK1, P<0.01; KYSE30-CCNA2, P<0.01; KYSE510-CDK1, P<0.01; KYSE510-CCNA2, P<0.01; one-way ANOVA followed by Dunnett's post hoc test; Fig. 3B and C).

*SNRPB knockdown promotes ESCC cell apoptosis*. The potential effects of SNRPB knockdown on apoptosis were determined in KYSE30 and KYSE510 cell lines. Results of the present study revealed a significant increase in the percentage of apoptotic cells in the SNRPB knockdown cells compared with shNC in both cell lines (Fig. 4A). As shown in Fig. 4A, flow cytometry analysis revealed that SNRPB knockdown led to a significant increase in the percentage of apoptotic cells in both KYSE30 and KYSE510 cell lines (KYSE30, P<0.001; KYSE510, P<0.001; determined by one-way ANOVA followed by Dunnett's post hoc test). To explore the molecular basis for this pro-apoptotic effect, the expression levels of key apoptosis-regulating proteins, Bax and Bcl-2, were determined by western blot analysis. Results of the present study revealed that shSNRPB1#1 shRNA-mediated SNRPB knockdown led to

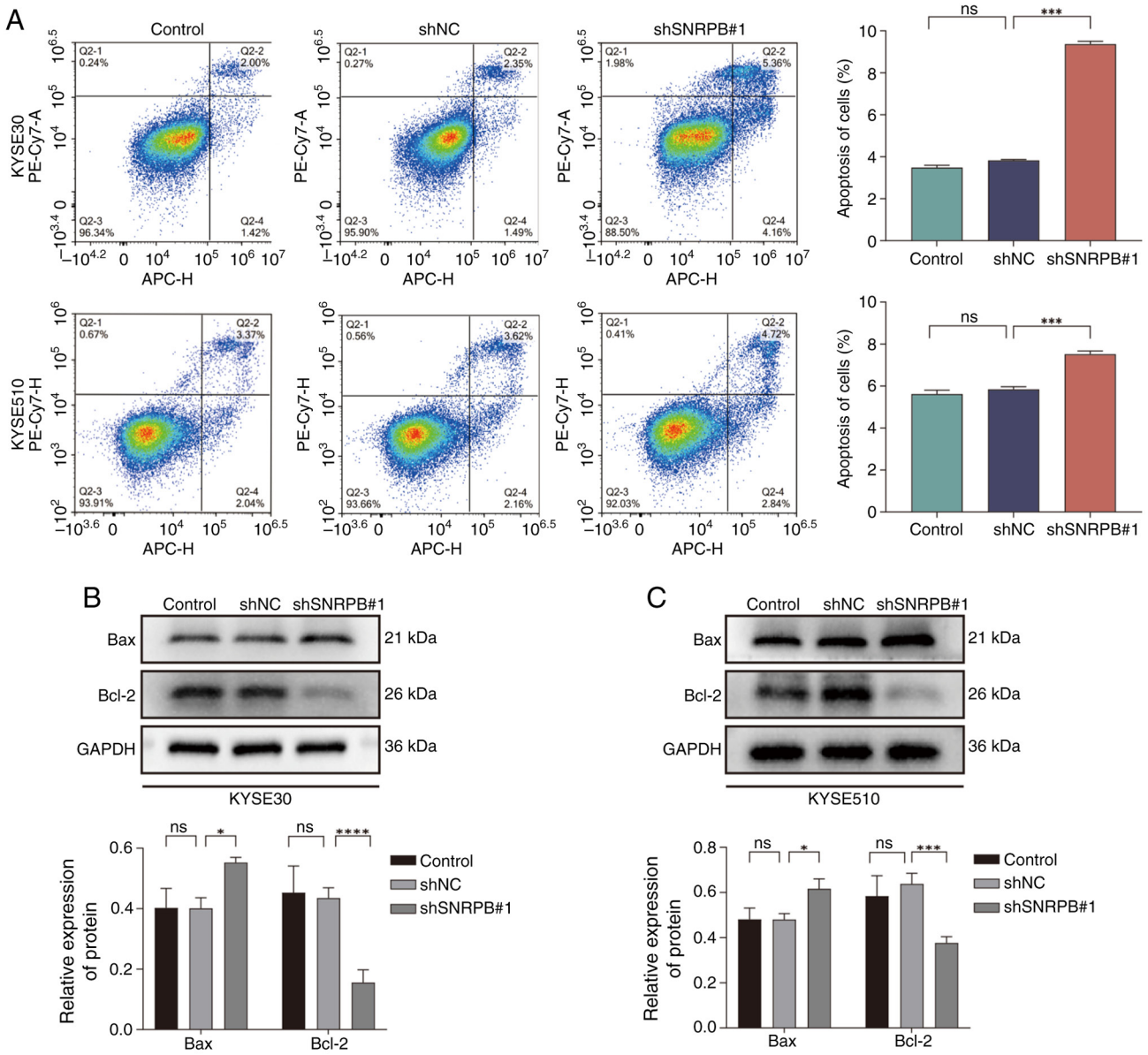


Figure 4. Silencing SNRPB induces esophageal squamous cell carcinoma cell apoptosis. (A) Proportion of apoptotic KYSE30 and KYSE510 cells after transduction with lentiviral constructs. (B) Apoptosis-related protein expression in KYSE30 cells. (C) Apoptosis-related protein expression in KYSE510 cells. Data are presented as the mean ± standard deviation. Statistical significance was determined using Student's t-test. $^{*}$P<0.05, $^{***}$P<0.001, $^{****}$P<0.0001; one-way ANOVA followed by Dunnett's post hoc test. shRNA, short hairpin RNA; ns, non-significant; NC, negative control; SNRPB, small nuclear ribonucleoprotein polypeptide B.

significant upregulation of Bax and significant downregulation of Bcl-2 protein expression levels, compared with those in the shNC group (KYSE30-Bax, P<0.001; KYSE30-Bcl-2, P<0.001; KYSE510-Bax, P<0.001; KYSE510-Bcl-2, P<0.001; one-way ANOVA followed by Dunnett's post hoc test; Fig. 4B and C).

## Discussion

Results of previous studies have indicated that SNRPB is overexpressed in a variety of tumor types and is implicated in tumor progression (15,16,26). However, its specific role in ESCC has remained largely unexplored. To the best of our knowledge, the present study is the first to demonstrate the oncogenic role of SNRPB in ESCC through an integrated approach of bioinformatic analysis, clinical validation and *in vitro* mechanistic investigations. The findings of the present study revealed high expression of SNRPB in ESCC tissues and revealed that its knockdown may inhibit cell proliferation and promote apoptosis.

Dysregulated alternative pre-mRNA splicing represents a hallmark molecular characteristic of the majority of neoplasms (27), and markedly contributes to the progression of ESCC and other malignancies (24). SNRPB, a core component of the spliceosome, carries out a key role in this process by mediating alternative splicing and regulating gene expression (18,28-30). The oncogenic role of SNRPB is supported by previous studies that reported high SNRPB expression levels in cervical (15), liver (19) and ovarian cancer (18), as well as its ability to promote proliferation in various cellular contexts (13). Consistent with these reports, the present

study demonstrated that SNRPB knockdown reduced the proliferation of ESCC cells. This anti-proliferative effect is mechanistically underpinned by two key events. First, arrest in the G2/M phase of the cell cycle was observed (31), which associated with the downregulation of key regulatory proteins CDK1 and Cyclin A2 (32-34). Second, SNRPB knockdown promoted apoptosis, which was associated with a disruption of the key balance between pro- and anti-apoptotic proteins, as evidenced by the upregulation of Bax (35) and concomitant downregulation of Bcl-2 (25).

Mechanistically, while the present study revealed that SNRPB depletion leads to these downstream molecular changes (including CDK1/Cyclin A2 downregulation and the Bax/Bcl-2 shift), the precise upstream link remains to be fully elucidated. As a core component of the spliceosome, the primary function of SNRPB is to regulate pre-mRNA splicing (10,18). It is plausible that the observed phenotypes are a consequence of altered alternative splicing of key regulatory genes. For example, previous studies in cervical and thyroid cancer have associated SNRPB to the inhibition of the tumor suppressor p53 (15,16). Given that p53 is a master regulator of both the G2/M checkpoint and the intrinsic apoptosis pathway (via Bax), it is a potential candidate for being a downstream target of SNRPB-mediated splicing in ESCC. Future research should aim to identify the specific splicing events regulated by SNRPB to fully unravel its oncogenic signaling network

However, the present study has several limitations that must be acknowledged. First, and most importantly, the findings of the present study are based entirely on *in vitro* experiments. *In vivo* validation using animal models, such as xenograft studies, is required to confirm the clinical relevance of the results. Second, the present study focused on cell proliferation and apoptosis; the potential role of SNRPB in other cancer hallmarks, such as migration and invasion, was not investigated. Third, a survival analysis was not performed on the institutional cohort of 50 patients due to the limited sample size and short follow-up period. Finally, a formal power analysis was not conducted prior to the present study to determine the optimal clinical sample size; the sample size was based on patient availability. This should be considered when interpreting the clinical correlation results. Future studies are therefore needed to address these limitations and build upon the findings of the present study.

The clinical implications of these findings may hold relevance. First, the correlation between high SNRPB expression and poor overall survival, as demonstrated in the TCGA cohort, substantiates its potential as a prognostic biomarker. Patients with SNRPB-high tumors could potentially be stratified for more aggressive treatment regimens or closer monitoring. Second, the results highlight SNRPB as a potential therapeutic target. Targeting the spliceosome machinery is an emerging anti-cancer strategy (26-28), and several small-molecule inhibitors of splicing are under clinical investigation. These findings suggest that patients with ESCC that have tumors overexpressing SNRPB might be particularly vulnerable to such therapies. Furthermore, given that altered splicing is frequently associated with treatment resistance (13,17,29,36), future studies should explore whether SNRPB contributes to chemoresistance in ESCC, potentially opening avenues for combination therapies.

Collectively, the molecular analyses of the present study demonstrated that SNRPB knockdown impacts key proteins involved in cell cycle progression and apoptosis, such as CDK1, Cyclin A2, Bax and Bcl-2. These findings suggested that SNRPB may play a key role in ESCC progression and may exhibit potential as a biomarker for clinical applications in ESCC.

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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

XFW contributed to conception and design of the study, project administration, funding acquisition, analysis and interpretation of clinical and experimental data, writing-reviewing and editing of the manuscript. HYG contributed to acquisition of experimental data, data curation, formal analysis, writing-original draft of the manuscript. JZ, GBH and YT contributed to acquisition of experimental data, visualization, investigation. LYL and ZCL contributed to conception of the study, resources, sample collection. XRC and XBW contributed to validation, formal analysis and interpretation of experimental data. All authors read and approved the final manuscript. XFW and HYG confirm the authenticity of all the raw data.

## Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, Sichuan, China (approval no. 2023ER344-1). Due to the retrospective nature of the present study and the anonymization of patient data, the Medical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, Sichuan, China waived the need of obtaining informed consent.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References


1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Siegel RL, Miller KD, Wagle NS and Jemal A: Cancer statistics, 2023. CA Cancer J Clin 73: 17-48, 2023.
3. Pennathur A, Gibson MK, Jobe BA and Luketich JD: Oesophageal carcinoma. Lancet 381: 400-412, 2013.
4. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin 69: 7-34, 2019.
5. Abnet CC, Arnold M and Wei WQ: Epidemiology of esophageal squamous cell carcinoma. Gastroenterology 154: 360-373, 2018.
6. Koshy M, Esiashvilli N, Landry JC, Thomas CR Jr and Matthews RH: Multiple management modalities in esophageal cancer: Epidemiology, presentation and progression, work-up, and surgical approaches. Oncologist 9: 137-146, 2004.
7. Huang FL and Yu SJ: Esophageal cancer: Risk factors, genetic association, and treatment. Asian J Surg 41: 210-215, 2018.
8. Nilsen TW and Graveley BR: Expansion of the eukaryotic proteome by alternative splicing. Nature 463: 457-463, 2010.
9. Wu J, Lu F, Yu B, Wang W and Ye X: The oncogenic role of SNRPB in human tumors: A pan-cancer analysis. Front Mol Biosci 9: 994440, 2022.
10. Bacrot S, Doyard M, Huber C, Alibeu O, Feldhahn N, Lehalle D, Lacombe D, Marlin S, Nitschke P, Petit F, *et al*: Mutations in SNRPB, encoding components of the core splicing machinery, cause cerebro-costo-mandibular syndrome. Hum Mutat 36: 187-190, 2015.
11. Lynch DC, Revil T, Schwartzentruber J, Bhoj EJ, Innes AM, Lamont RE, Lemire EG, Chodirker BN, Taylor JP, Zackai EH, *et al*: Disrupted auto-regulation of the spliceosomal gene SNRPB causes cerebro-costo-mandibular syndrome. Nat Commun 5: 4483, 2014.
12. Knill C, Henderson EJ, Johnson C, Wah VY, Cheng K, Forster AJ and Itasaki N: Defects of the spliceosomal gene SNRPB affect osteo- and chondro-differentiation. FEBS J 291: 272-291, 2024.
13. Liu N, Wu Z, Chen A, Wang Y, Cai D, Zheng J, Liu Y and Zhang L: SNRPB promotes the tumorigenic potential of NSCLC in part by regulating RAB26. Cell Death Dis 10: 667, 2019.
14. Wang X, Zhang H, Guo Z, Wang J, Lu C, Wang J, Jin R and Mo Z: SNRPB promotes the progression of hepatocellular carcinoma via regulating cell cycle, oxidative stress, and ferroptosis. Aging (Albany NY) 16: 348-366, 2024.
15. Zhu L, Zhang X and Sun Z: SNRPB promotes cervical cancer progression through repressing p53 expression. Biomed Pharmacother 125: 109948, 2020.
16. Deng Y, Li X, Jiang W and Tang J: SNRPB promotes cell cycle progression in thyroid carcinoma via inhibiting p53. Open Med (Wars) 17: 1623-1631, 2022.
17. Li Y, Chen Z, Xiao H, Liu Y, Zhao C, Yang N, Yuan C, Yan S and Li P: Targeting the splicing factor SNRPB inhibits endometrial cancer progression by retaining the POLD1 intron. Exp Mol Med 57: 420-435, 2025.
18. Li Y, Chen Z, Peng J, Yuan C, Yan S, Yang N, Li P and Kong B: The splicing factor SNRPB promotes ovarian cancer progression through regulating aberrant exon skipping of POLA1 and BRCA2. Oncogene 42: 2386-2401, 2023.
19. Peng N, Li J, He J, Shi X, Huang H, Mo Y, Ye H, Wu G, Wu F, Xiang B, *et al*: c-Myc-mediated SNRPB upregulation functions as an oncogene in hepatocellular carcinoma. Cell Biol Int 44: 1103-1111, 2020.
20. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 45: W98-W102, 2017.
21. Therneau T: A Package for Survival Analysis in R. R Foundation for Statistical Computing, Vienna, Austria2024.
22. Dumitru CA, Bankfalvi A, Gu X, Zeidler R, Brandau S and Lang S: AHNAK and inflammatory markers predict poor survival in laryngeal carcinoma. PLoS One 8: e56420, 2013.
23. Bowker AH: A test for symmetry in contingency tables. J Am Stat Assoc 43: 572-574, 1948.
24. Zhang Y, Qian J, Gu C and Yang Y: Alternative splicing and cancer: A systematic review. Signal Transduct Target Ther 6: 78, 2021.
25. Maulik N, Engelman RM, Rousou JA, Flack JE III, Deaton D and Das DK: Ischemic preconditioning reduces apoptosis by upregulating anti-death gene Bcl-2. Circulation 100: II369-II375, 1999.
26. Zhan YT, Li L, Zeng TT, Zhou NN, Guan XY and Li Y: SNRPB-mediated RNA splicing drives tumor cell proliferation and stemness in hepatocellular carcinoma. Aging (Albany NY) 13: 537-554, 2020.
27. Bradley RK and Anczuków O: RNA splicing dysregulation and the hallmarks of cancer. Nat Rev Cancer 23: 135-155, 2023.
28. Gray TA, Smithwick MJ, Schaldach MA, Martone DL, Graves JA, McCarrey JR and Nicholls RD: Concerted regulation and molecular evolution of the duplicated SNRPB'/B and SNRPN loci. Nucleic Acids Res 27: 4577-4584, 1999.
29. Zheng Y, Niu X, Xue W, Li L, Geng Q, Fan Z and Zhao J: The role of alternative splicing factors hnRNP G and Fox-2 in the progression and prognosis of esophageal cancer. Dis Markers 2022: 3043737, 2022.
30. Wu H, Zheng J, Deng J, Zhang L, Li N, Li W, Li F, Lu J and Zhou Y: LincRNA-uc002yug.2 involves in alternative splicing of RUNX1 and serves as a predictor for esophageal cancer and prognosis. Oncogene 34: 4723-4734, 2015.
31. Evan GI and Vousden KH: Proliferation, cell cycle and apoptosis in cancer. Nature 411: 342-348, 2001.
32. Lohberger B, Leithner A, Stuendl N, Kaltenegger H, Kullich W and Steinecker-Frohnwieser B: Diacerein retards cell growth of chondrosarcoma cells at the G2/M cell cycle checkpoint via cyclin B1/CDK1 and CDK2 downregulation. BMC Cancer 15: 891, 2015.
33. Ma Q: MiR-219-5p suppresses cell proliferation and cell cycle progression in esophageal squamous cell carcinoma by targeting CCNA2. Cell Mol Biol Lett 24: 4, 2019.
34. Fischer M, Quaas M, Steiner L and Engeland K: The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. Nucleic Acids Res 44: 164-174, 2016.
35. Schellenberg B, Wang P, Keeble JA, Rodriguez-Enriquez R, Walker S, Owens TW, Foster F, Tanianis-Hughes J, Brennan K, Streuli CH and Gilmore AP: Bax exists in a dynamic equilibrium between the cytosol and mitochondria to control apoptotic priming. Mol Cell 49: 959-971, 2013.
36. Wang E and Aifantis I: RNA splicing and cancer. Trends Cancer 6: 631-644, 2020.