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RESEARCH ARTICLE

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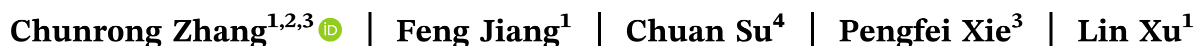
Upregulation of long noncoding RNA SNHG20 promotes cell growth and metastasis in esophageal squamous cell carcinoma via modulating ATM-JAK-PD-L1 pathway



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Abstract

Increasing evidence have proved that long noncoding RNAs (lncRNAs) play significant roles in tumorigenesis and development of various cancers. However, the effect of small nucleolar RNA host gene 20 (SNHG20) on the progression of esophageal squamous cell carcinoma (ESCC) remains to be discovered. Herein, we aim to find out the function and the possible mechanism of SNHG20 in ESCC progression. In our study, we demonstrate that SNHG20 is markedly upregulated in ESCC tissues and cell lines. Besides, the level of SNHG20 is closely associated with tumor size, lymph node metastasis, TNM stage, and tumor grade. In addition, SNHG20 level is an independent predictor for clinical outcomes of ESCC patients. Then the gain- and loss-of-function assays reveal that SNHG20 overexpression promotes cell proliferation, migration, invasion, and epithelial-mesenchymal transition as well as represses apoptosis, whereas depletion of SNHG20 exhibits opposite effects. Moreover, we uncover that SNHG20 modulates the expression of ataxia telangiectasia–mutated kinase (p-ATM), p-JAK1/2, and programmed cell death 1 ligand 1 (PD-L1) in ESCC cells and ATM upregulation restores the suppressive effect of SNHG20 inhibition on ESCC progression. Therefore, we conclude that SNHG20 serves as a carcinogen in ESCC by promoting growth and metastasis via ATM-JAK-PD-L1 pathway, supplying a possibly effective therapeutic target for ESCC.

KEYWORDS

ATM-JAK-PD-L1 pathway, epithelial-mesenchymal transition, esophageal squamous cell carcinoma, metastasis, proliferation, small nucleolar RNA host gene 20

1 | INTRODUCTION

Human esophageal cancer (EC), ranking third in the most common digestive system neoplasms, has high mortality all over the world.[1] Patients with EC suffer from a short survival life because of the high recurrence and metastasis.[2] Compared to esophageal adenocarcinoma, esophageal squamous cell carcinoma (ESCC) that accounts for more than 90% of EC cases exhibits evident etiological and pathological features.[3] Despite advances in the treatment of ESCC, such as surgery, chemotherapy, radiotherapy, and their combining therapy, the prognosis of patients is still poor.[4-7] Thus, it is urgently necessary to find out molecular mechanism of ESCC so as to search for novel effective prognostic biomarker and therapeutic schemes.

Long noncoding RNA (lncRNA) is identified as a class of RNAs (>200 nt) without protein-coding capacity due to

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the deficiency of open reading frame (ORF).[8] In the last decade, lncRNAs have been proved to participate in a variety of vital biological processes, including tumorigenesis.[9] Besides, lncRNAs are also largely implicated in ESCC.[10,11] For instance, silencing of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) by microRNA-101 (miR-101) and miR-217 inhibits proliferation, migration, and invasion of ESCC cells.[12] Small nucleolar RNA host gene 20 (SNHG20; Gen-Bank Accession ID NR_027058.1) located at 17q25.2 is first discovered in hepatocellular carcinoma (HCC)[13] and has been proved to be of great importance in several cancers, such as cervical cancer,[14] breast cancer,[15] ovarian cancer,[16] and colorectal cancer.[17] However, its role in ESCC remains undiscovered.

Epithelial-mesenchymal transition (EMT) has been well-verified to be a probable mechanism of tumor invasion and metastasis.[18] In the progression of EMT, epithelial cells gradually lose its intrinsic structure and basement membranes, the newly clustered cells grow faster and become more invasive.[19] In addition, aberrant expression of EMT markers, such as E-cadherin, N-cadherin, and vimentin, plays a key role in tumor metastasis of various malignancies, including ESCC.[20-22] Moreover, ZEB1 is also a key regulator of EMT whose expression changes in numerous tumors.[23]

In this study, the role of SNHG20 in ESCC and the underlying mechanism are investigated.

2 | MATERIALS AND METHODS

2.1 | Human tissue specimens

This study is approved by the Ethics Committee of Affiliated Cancer Hospital of Nanjing Medical University. Total of 80 pairs of tumor tissues and corresponding nontumor tissues obtained from patients with primary ESCC are involved and informed consent was signed by all patients concerning the use of these clinical materials in this study. All patients that accepted no any other treatments before have undergone surgical treatment at Affiliated Cancer Hospital of Nanjing Medical University.

2.2 | Cell culture

The normal human esophageal epithelial cell line (Het-1A) is obtained from Jenniobio Biotechnology (Guangzhou, China), and four ESCC cell lines (KYSE450, KYSE150, EC9706, and EC109) are purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and Cancer Institute and Hospital, Chinese Academy of Medical Sciences (Beijing, China). All of the cells are cultured in RPMI-1640 medium (HyClone, Logan, UT) containing 10% fetal bovine serum (FBS; HyClone) and kept under a humidified atmosphere with 5% CO_2 at 37°C.

2.3 | RNA isolation and quantitative reverse-transcription polymerase chain reaction

Total RNA is isolated from tissues or cells using TRIzol Reagent (Takara, Dalian, China) under the manufacturer's instructions. Then RNA is transcribed-reversely into complementary DNA using a Reverse Transcription Kit (Takara) according to the manufacturer's protocols. Real-time polymerase chain reaction (PCR) analysis is conducted with SYBR Premix Ex Taq (Takara). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is used as the endogenous control. The quantitative reverse-transcription (qRT) PCR assays are performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Relative changes are counted with $2^{-\Delta\Delta C_t}$ method. The sequences of primers used here are listed below: SNHG20, forward: 5′-ATGGCTATAAATA GATACACGC-3′, reverse: 5′-GGTACAAACAGGGAGG GA-3′; GAPDH, forward: 5′-CGGAGTCAACGGATTTG GTCGTAT-3′, reverse: 5′-AGCCTTCTCCATGGTGGTG AAGAC-3′.

2.4 | Cell transfection

Specific short hairpin RNA (shRNA) targeting SNHG20 (shSNHG20) and its corresponding negative control shRNA (shCtrl) are purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Overexpression of SNHG20 is accomplished through transfection with pcDNA3.1/SNHG20, while the empty vector acts as a control. Transfection for 48 hours later, the cells are harvested and detected. Cell transfections are practiced using Lipofectamine 3000 Transfection Reagent (Invitrogen, Carlsbad, CA) on the basis of the manufacturer's protocols.

2.5 | MTT assay

Cell viability is evaluated by using MTT (Sigma) assay and determined by cell survival rates at 0, 24, 48, 72, and 96 hours after transfection. First, all cells are placed in 96-well plates (1×10^4 cells/well) and maintained in an incubator at 37°C with 5% CO_2. After that, MTT is added into each well at a density of 0.5 mg/mL and the plates are further incubated for 4 hours. Then the supernatant is wiped off and dimethyl sulfoxide (DMSO) is used to dissolve the formed formazan. Absorbance at 490 nm is measured using Microplate Reader (Molecular Devices, i3).

2.6 | Colony formation assays

The SNHG20 silenced or overexpressed ESCC cells at a density of 1000 cells/well are added into six-well plates and cultured in Dulbecco's modified Eagle's medium containing 10% FBS at 37°C. After 2 weeks, cells are fixed with methanol and stained using 1% crystal violet dye after washing with phosphate-buffered saline. And the number of colonies is counted manually. Experiments are repeated for triplicate.

2.7 | Flow cytometry analysis

First, KYSE450 cells transfected with shSNHG20 or shCtrl and Het-1A cells transfected with pcDNA3.1/SNHG20 or pcDNA3.1 vector are seeded in six-well plates. After 48 hours of incubation, cells are collected and then cell apoptosis rates are determined using flow cytometry after treating with propidium iodide (PI) and annexin V- fluorescein isothiocyanate (BD Bioscience, San Jose, CA). Cell apoptosis assays are performed in triplicate.

2.8 | Cell migration and invasion assays

Cell migration and invasion abilities are determined by transwell cell culture chambers (BD Matrigel Invasion Chamber; BD Biosciences) with 8 μm pore size under the manufacturer's recommendations. For migration assays, 1×10^5 cells in 200 μL serum-free medium are added into the upper chamber, and the lower chamber is added with 500 μL medium containing 10% FBS. After incubation for 24 hours, cells migrated to the lower chamber are fixed and stained using crystal violet. Cells on the upper surface are removed using a cotton swap. For invasion assay, all steps are the same with that in migration assay except that the 1×10^5 cells are seeded in the upper chamber which has been coated with Matrigel before. The number of migrated or invaded cells at five random fields is counted under a microscope and represented as the average per field.

2.9 | Western blot analysis

Protein lysates from cells are isolated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then shifted to nitrocellulose membrane. Then the membranes are blocked with 5% nonfat milk and incubated with primary antibodies overnight at 4°C. The primary antibodies used in this study are as below: E-cadherin, ZEB1, N-cadherin, and vimentin (1:1000, above antibodies from Cell Signaling Technology, MA); phospho-ataxia telangiectasia–mutated kinase (p-ATM), Programmed cell death 1 ligand 1 (PD-L1), p-JAK1/2, and GAPDH (1:1000, all from Thermo Fisher Scientific, Inc, Waltham, MA). GAPDH is used as an endogenous control. Then the membranes are incubated with horseradish peroxidase–conjugated (HRP) secondary antibody (1:10 000, Santa Cruz Biotechnology) for 1 hour after washing. The signals are visualized by the ECL detection system (Thermo Fisher Scientific, Inc) and the bands are quantitated using Quantity One software (BioRad, Hercules, CA). Also, the ATM inhibitor is listed here, CP466722 (S2245; Selleck Chemicals, Houston).

2.10 | Statistical methods

Data are obtained from at least triplicate independent experiments and expressed as mean ± standard deviation. Differences between the two groups are evaluated with the student *t* test. Overall survival rates are determined by the Kaplan-Meier analysis and the log-rank test, and clinical data is analyzed by univariate and multivariate Cox proportional hazards models. All statistical analyses are conducted using SPSS 17.0 software, and $P < 0.05$ is considered to have statistical significance.

3 | RESULTS

3.1 | SNHG20 is upregulated in human ESCC tissues and cell lines

To investigate the function of SNHG20 in ESCC progression, we firstly examine SNHG20 expression in 80 paired ESCC tissues and corresponding para-carcinoma tissues. Then the obvious high expression of SNHG20 in ESCC tissues compared with adjacent nontumor tissues is represented by qRT-PCR results (Figure 1A). To further make sure the upregulation of SNHG20 in ESCC, we detect the SNHG20 expression in normal Het-1A and ESCC cell lines (KYSE450, KYSE150, EC9706, and EC109) using RT-qPCR analysis. As shown in Figure 1B, all the four ESCC cell lines exhibit higher levels of SNHG20 in contrast to Het-1A cells, among which SNHG20 expression in KYSE450 cells is the highest. These results indicate that SNHG20 might act as an oncogene in ESCC progression.

3.2 | SNHG20 is associated with clinicopathological characteristics and its upregulation related to poor clinical outcomes in ESCC

To further investigate the impact of SNHG20 on ESCC, the association between SNHG20 expression and

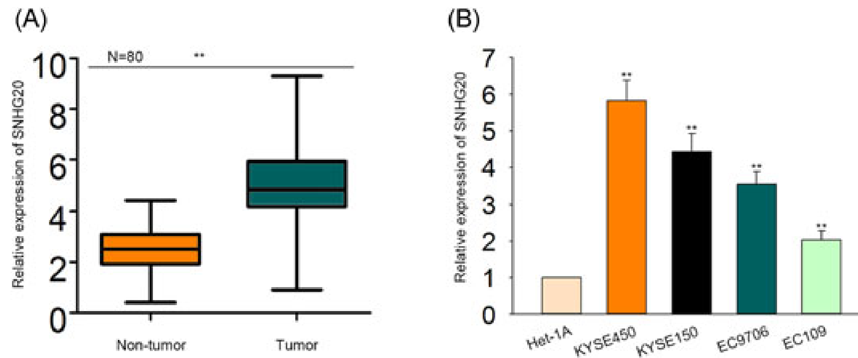

FIGURE 1 SNHG20 is highly expressed in ESCC tissues and cell lines. A, The expression of SNHG20 tested by quantitative reverse-transcription polymerase chain reaction is upregulated in ESCC tissues ($n = 80$) compared with adjacent nontumor tissues ($n = 80$). And GAPDH is the normalized control. B, Relative expression of SNHG20 in a normal human esophageal epithelial cell line Het-1A and four ESCC cell lines (KYSE450, KYSE150, EC9706, and EC109). All of the cell lines are analyzed from data of three-independent experiments. *$P < 0.05$ and **$P < 0.01$. ESCC, esophageal squamous cell carcinoma; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Het-1A, human esophageal epithelial cell line; SNHG20, small nucleolar RNA host gene 20

clinicopathological characteristics of ESCC patients is analyzed. First, all of the 80 ESCC patients are divided into two groups, among which 43 are with low SNHG20 expression while 37 with high SNHG20 level. As presented in Table 1, the level of SNHG20 in patients with ESCC is closely associated with tumor size, lymph node metastasis, TNM stage, and tumor grade. In addition, the results of Cox regression analysis revealed that only the level of SNHG20 and TNM stage could serve as the independent prognostic markers for ESCC patients (Table 2). Moreover, the Kaplan-Meier curve suggests that higher SNHG20

TABLE 1 Correlation between SNHG20 expression and clinical features (n = 80)

	SNHG20 expression		
Variables	**Low**	**High**	***P* value**
Age			
<60	13	13	0.811
≥60	30	24	
Sex			
Male	8	9	0.591
Female	35	28	
Location			
Lower	17	13	0.817
Middle-upper	26	24	
Tumor size			
≤4	25	10	0.007
>4	18	27	
Lymph node metastasis			
N0	26	11	0.007
N1-N3	17	26	
TNM Stage			
I-II	25	7	0.001
III-IV	18	30	
Tumor grade			
G1	25	11	0.014
G2-G3	18	26	

Low/high by the sample median. Pearson χ^2 test. $P < 0.05$ is considered to be statistically significant.

TABLE 2 Multivariate analysis of prognostic parameters in patients with esophageal squamous cell carcinoma by Cox regression analysis

Variables	***P* value**
Age	
<60	0.979
≥60	
Sex	
Male	0.735
Female	
Location	
Lower	0.334
Middle-upper	
Tumor Size	
≤4	0.300
>4	
Lymph node metastasis	
N0	0.084
N1-N3	
TNM stage	
I-II	0.026*
III-IV	
Tumor grade	
G1	0.621
G2-G3	
SNHG20 level	
Low	0.013*
High	

Proportional hazards method analysis reveals a positive, independent prognostic importance of SNHG20 expression ($P = 0.013$). *$P < 0.05$ is considered to be statistically significant.

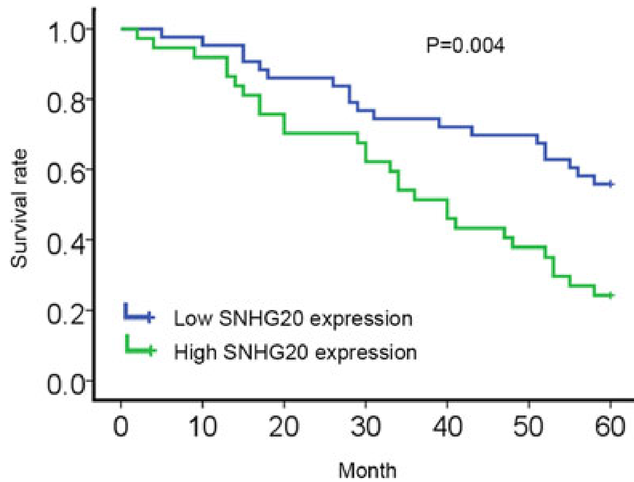

FIGURE 2 SNHG20 expression is related to ESCC prognosis. The ESCC patients are classified into two groups based on low (n = 43) or high (n = 37) SNHG20 expression level. Kaplan-Meier curves indicate that high SNHG20 expression is related to poor overall survival of patients with ESCC. ESCC, esophageal squamous cell carcinoma; SNHG20, small nucleolar RNA host gene 20

expression in ESCC patients is strongly correlated with poorer overall survival (Figure 2). These data suggest that SNHG20 may be functional in ESCC progression, implying a novel biomarker for the prognosis of ESCC patients.

3.3 | SNHG20 affects biological behaviors of ESCC cells

To explore the biological functions of SNHG20 in the development and progression of ESCC, the loss- and gain-of-function assay is carried out by silencing SNHG20 expression in KYSE450 cells using targeted shRNAs and overexpressing it in Het-1A cells using pcDNA3.1 vectors, respectively. As illustrated in Figure 3A, SNHG20 is apparently inhibited in KYSE450 cells and markedly overexpressed in Het-1A cells. MTT assays demonstrate that cell viability is strikingly impaired in shSNHG20 transfected KYSE450 cells but significantly enhanced in pcDNA3.1/SNHG20 transfected Het-1A cells (Figure 3B). Consistently, SNHG20 knockdown represses the colony forming ability of KYSE450 cells while SNHG20 overexpression increases that of Het-1A cells (Figure 3C). Furthermore, cell apoptosis rate is notably increased in KYSE450 cells under SNHG20 silence but significantly decreased in Het-1A cells upon SNHG20 upregulation (Figure 3D). Collectively, these results indicate that SNHG20 promotes cell growth in ESCC.

3.4 | SNHG20 facilitates metastasis in ESCC by promoting EMT

To figure out whether dysregulation of SNHG20 contributes to tumor metastasis of ESCC, we explore

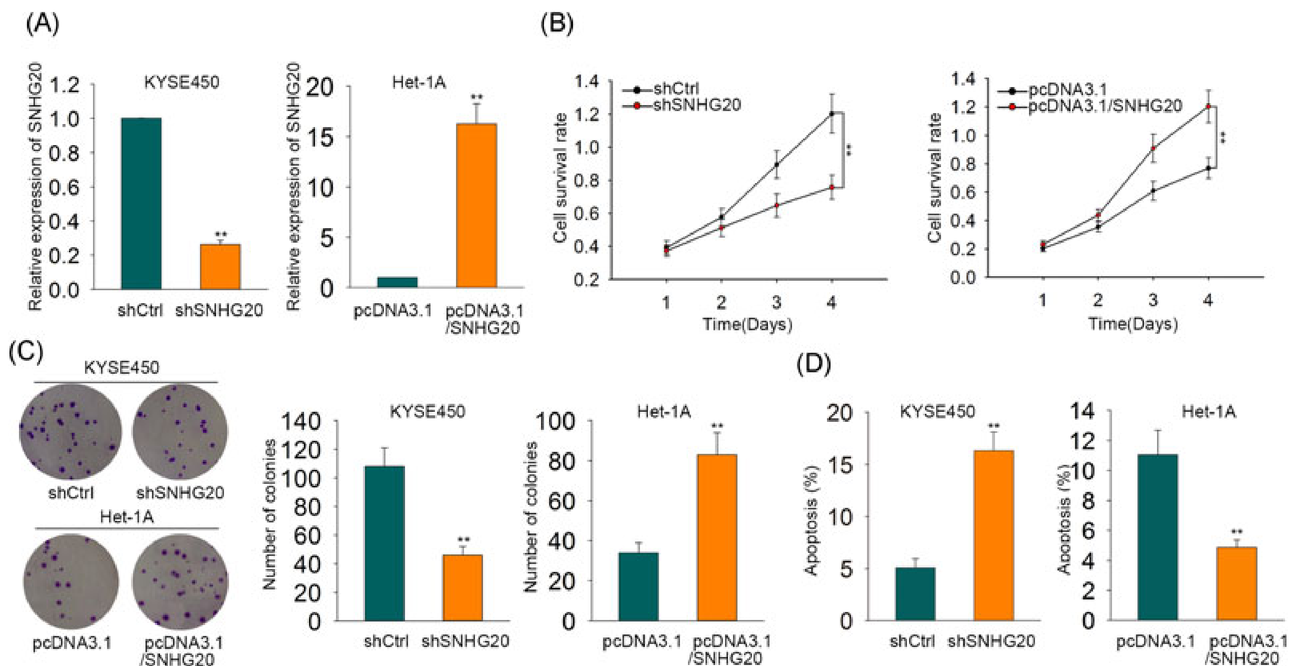

FIGURE 3 Effect of SNHG20 on ESCC cell proliferation and apoptosis in vitro. A, Quantitative reverse-transcription polymerase chain reaction results of SNHG20 expression in KYSE450 and Het-1A cells after different transfections. B, MTT assay is used to detect cell survival rate in shSNHG20 transfected KYSE450 cells and pcDNA3.1/SNHG20 transfected Het-1A cells. C, Cell proliferation ability in KYSE450 cells under SNHG20 silence and Het-1A cells upon SNHG20 overexpression is determined by colony formation assay. D, Flow cytometry analysis is utilized to evaluate the proportion of apoptotic cells in ESCC cells after SNHG20 silence or overexpression. Data is shown as the mean ± standard deviation. All experiments are carried out in triplicate. *$P < 0.05$ and **$P < 0.01$. ESCC, esophageal squamous cell carcinoma; Het-1A, human esophageal epithelial cell line; shCtrl, negative control short hairpin RNA; shSNHG20, short hairpin RNA targeting SNHG20; SNHG20, small nucleolar RNA host gene 20

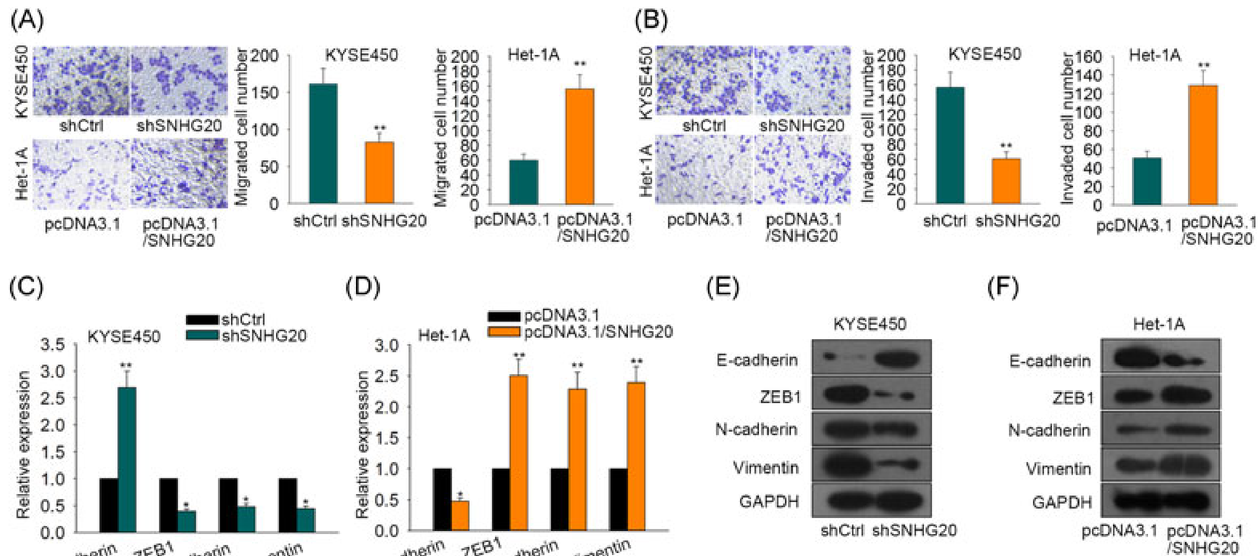

FIGURE 4 SNHG20 facilitates metastasis in ESCC by promoting EMT. A, Transwell assays results of migratory abilities in SNHG20-silenced KYSE450 cells and SNHG20 overexpressed Het-1A cells. B, Transwell assay for the assessment of cell invasion in KYSE450 cells after transfection with shCtrl or shSNHG20 or in Het-1A cells after transfection with pcDNA3.1 or pcDNA3.1-SNHG20. C and D, Relative messenger RNA expression of E-cadherin, ZEB1, N-cadherin, and vimentin are examined by quantitative reverse-transcription polymerase chain reaction in ESCC cells under different transfections. E and F, Relative protein levels of E-cadherin, ZEB1, N-cadherin, and vimentin are examined by Western blot analysis in above ESCC cells. Results are exhibited as the mean ± standard deviation on the basis of three-independent experiments, $*P < 0.05$ and $**P < 0.01$. EMT, epithelial-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; Het-1A, human esophageal epithelial cell line; shCtrl, negative control short hairpin RNA; shSNHG20, short hairpin RNA targeting SNHG20; SNHG20, small nucleolar RNA host gene 20

its effect on cell migration and invasion using transwell assays. As determined in Figure 4A and 4B, SNHG20 silence leads to evident repression on cell migration of KYSE450 cells, whereas SNHG20 overexpression exhibits improved migratory ability in Het-1A cells. Similarly, cell invasive capacity is remarkably declined under SNHG20 silence but distinctly enhanced after SNHG20 upregulation (Figure 4C and 4D). EMT has been increasingly reported to be involved in cell invasion and metastasis.[18] Based on this, we wonder whether SNHG20 affects the EMT process in ESCC. As revealed in Figure 4E, knockdown of SNHG20 greatly elevates the expression of epithelial marker E-cadherin expression while evidently decreases the expression of the mesenchymal markers N-cadherin and vimentin as well as that of ZEB1, a key regulator of EMT. On contrary, SNHG20 overexpression leads to reduced E-cadherin expression and strengthened levels of N-cadherin, vimentin, and ZEB1 (Figure 4F). Accordingly, the protein levels of EMT-related genes determined by Western blot analysis further confirm the results above (Figure 4G and 4H). Thus, we conclude that SNHG20 stimulates EMT process in ESCC cells to promote tumor metastasis in ESCC.

3.5 | SNHG20 facilitates ESCC cell growth and metastasis via regulating ATM-JAK-PD-L1 pathway

Recently, ATM-JAK-PD-L1 signaling pathway promotes EMT and metastasis of androgen-independent prostate cancer.[24] Additionally, ATM, JAK and PD-L1 have all been reported to participate in cell proliferation in human cancers.[25-27] Thus, we hypothesize that SNHG20 affects cell growth and metastasis in ESCC via ATM-JAK-PD-L1 pathway. To validate this hypothesis, we first examine whether SNHG20 has an impact on the levels of proteins involved in this pathway. As a result, we observe that the levels of p-ATM, p-JAK1/2, and PD-L1 are downregulated in KYSE450 cells under SNHG20 inhibition but upregulated in Het-1A cells after pcDNA3.1/SNHG20 transfection (Figure 5A and 5B), indicating that SNHG20 modulates the activation of ATM/JAK/PD-L1 pathway in ESCC cells.

To make further confirmation about the above findings, a pcDNA3.1 vector targeting ATM (pcDNA3.1/ATM) and its negative control (pcDNA3.1) are applied to perform rescue assays in SNHG20-silenced KYSE450 cells. First of all, we find that the transfection of pcDNA3.1/ATM or pcDNA3.1 has no influence on SNHG20 expression (Figure 5C). By contrast, cotransfection of pcDNA3.1/ATM

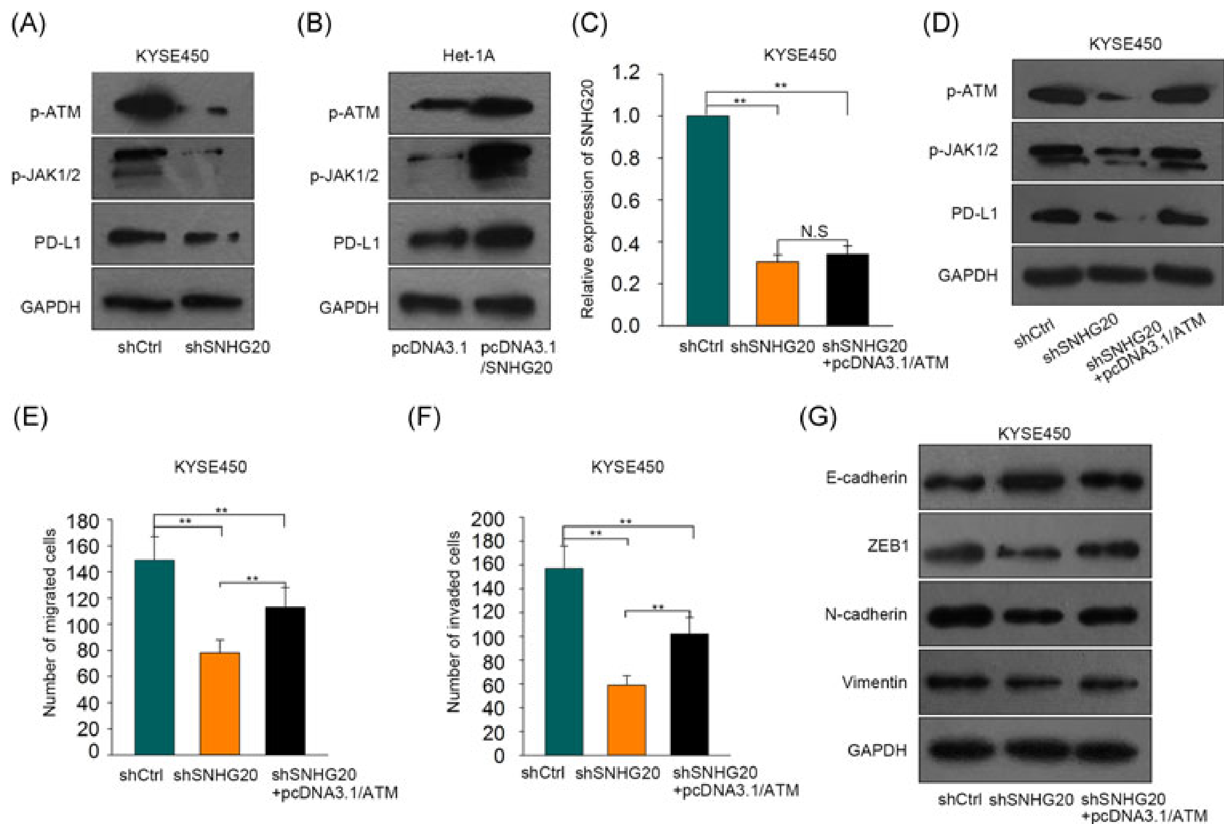

FIGURE 5 SNHG20 affects cellular processes in ESCC through ATM-JAK-PD-L1 pathway. A and B, Western blot analysis is used to detect the protein levels of p-ATM, p-JAK1/2, and PD-L1 after silencing or overexpressing SNHG20 in KYSE450 or Het-1A cells, respectively. And GAPDH acts as the normalized control. C, Relative SNHG20 expression detected by quantitative reverse-transcription polymerase chain reaction is not affected by pcDNA3.1 or pcDNA3.1/ATM in KYSE450 cells. D, Western blot analysis results of the changes of p-ATM, p-JAK1/2, and PD-L1 levels upon ATM upregulation in shSNHG20 transfected KYSE450 cells. E and F, The changes on cell migration and invasion capacities altered by cotransfection of pcDNA3.1 or pcDNA3.1/ATM are evaluated using transwell assays in SNHG20-silenced KYSE450 cells. G, The alterations in the protein levels of EMT-related genes after cotransfection of shSNHG20 and pcDNA3.1 or pcDNA3.1/ATM in KYSE450 cells are measured by Western blot analysis. Data is obtained from three-independent tests and represented as the mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$. ATM, ataxia telangiectasia–mutated kinase; EMT, epithelial-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Het-1A, human esophageal epithelial cell line; NS, non significant; PD-L1, programmed cell death 1 ligand 1; shCtrl, negative control short hairpin RNA; shSNHG20, short hairpin RNA targeting SNHG20; SNHG20, small nucleolar RNA host gene 20

results in promoted levels of p-ATM, p-JAK1/2, and PD-L1 in KYSE450 cells in comparison with the SNHG20-silenced group, validating that JAK1/2 and PD-L1 are the downstream factors of ATM (Figure 5D). Moreover, ectopic expression of ATM reverses the repressed capacities of cell migration and invasion caused by SNHG20 inhibition (Figure 5E and 5F). Furthermore, ATM upregulation causes remarkable lowered E-cadherin expression but the enhanced level of ZEB1, N-cadherin, and vimentin in SNHG20-suppressed KYSE450 cells (Figure 5G). Taken together, SNHG20 affects EMT and metastasis in ESCC by modulating ATM-JAK-PD-L1 pathway.

4 | DISCUSSION

ESCC is one of the most malignant tumors around the world, and patients with ESCC always have a poor 5-year survival.[28] In spite of improvements in the treatments of ESCC, local recurrences and distant metastases occur commonly and the overall survival of ESCC patients remains poor.[4] Hence, relevant studies on novel therapeutic targets or prognostic biomarkers for ESCC is essential.

LncRNAs are a class of transcripts that have been found increasingly to exert crucial roles in multiple cancers.[29] SNHG20 is an lncRNA that has been reported to act as an oncogene in several cancers.[13-17] For example, SNHG20 predicts a poor prognosis for HCC and promotes cell invasion by regulating the EMT.[30] Unsurprisingly, we also observed the upregulation of SNHG20 in ESCC tissues and cell lines. In addition, our study demonstrates that SNHG20 promotes cell proliferation, migration, and invasion as well as EMT in ESCC cells.

ATM is a significant modulator in DNA damage response through regulating target genes after its activation of serine phosphorylation, and the dysregulation of ATM contributes to the progression of many cancers.[31-33] For example, ATM depletion inhibits cell proliferation and migration in colon cancer.[26] Activation of the ATM-Snail pathway promotes breast cancer metastasis.[34] Recently, advances in the understanding of ATM signaling mechanisms in cancer and its inhibition as a strategy for cancer treatment have been made.[35] JAK signaling is a common oncogenic pathway which has been found to function in various human disease including cancer.[36,37] Sen et al have suggested that JAK kinase inhibition abrogates STAT3 activation and head and neck squamous cell carcinoma tumor growth.[27] Also, this signaling affects EMT in HCC.[38] PD-L1 functions as a prosurvival role in cancer,[39] which is also closely associated with EMT and metastasis in neoplasms.[25,40,41] Furthermore, PD-L1 has been verified to be associated with JAK activation.[42] Intriguingly, ATM-JAK-PD-L1 signaling pathway has been discovered to promote EMT and metastasis of androgen-independent prostate cancer.[24] In present study, we firstly uncover that SNHG20 modulates the levels of p-ATM, p-JAK1/2, and PD-L1 in ESCC cells. Therefore, we hypothesize that SNHG20 affects the biological processes in ESCC through this pathway, which is validated by subsequent rescue assays conducted under ATM overexpression.

All in all, SNHG20 serves as an oncogene in ESCC and its tumorigenesis-promoting role in ESCC is dependent on ATM-JAK-PD-L1 pathway. Our findings give a novel insight that SNHG20 can be an effective target for ESCC prognosis and treatment, although deeper research are still in need for its clinical application in the future.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Chunrong Zhang contributed to article writing. All authors were responsible for experimental design and proofread the final version of manuscript. Feng Jiang and Chuan Su devoted themselves to data collection. Pengfei Xie and Lin Xu were responsible for Statistics analysis.

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