Clinical Nutrition & Hospital Dietetics

Research Article

Clin Nutr Hosp Diet. 2024; 44(2): 01-12
DOI: 10.12873/0211-6057.44.02.221

SNAI1 as a biomarker for prognostic prediction and targeted therapy in lung squamous cell carcinoma

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Received date: 03-Jun-2024, Manuscript No. CNHD-24-137915; **Editor assigned date:** 05-Jun-2024, PreQC No. CNHD-24-137915 (PQ); **Reviewed date:** 19-Jun-2024, QC No. CNHD-24-137915; **Revised date:** 26-Jun-2024, Manuscript No. CNHD-24-137915 (R); **Published date:** 03-Jul-2024, DOI: 10.12873/0211-6057.44.02.221

ABSTRACT

Background: This study investigates the role and potential molecular mechanisms of the *SNAI1* gene in Lung Squamous Cell Carcinoma (LUSC) and its application in molecular targeted therapy.

Methods: Bioinformatics analysis, including TCGA, TPA, GSEA and CellMiner analysis, was conducted to analyze the expression levels of the *SNAI1* gene in normal and tumour tissues, their correlation with clinical outcomes and potential targeted drugs.

Results: In normal human tissues, *SNAI1* was significantly highly expressed in lung tissues compared to other tissues. However, in LUSC, its expression was significantly downregulated. High expression of *SNAI1* mRNA was associated with poor Overall Survival (OS) and Disease-Free Survival (DFS). The expression level of *SNAI1* mRNA was also associated with age, tumour size, lymph node metastasis and distant metastasis in LUSC patients. A nomogram was constructed to predict the survival of LUSC patients. Furthermore, high expression of the *SNAI1* protein in LUSC was associated with poor prognosis. The 5-year survival rate was 37% in the high expression group and 59% in the low expression group. The main subcellular localization of *SNAI1* protein in LUSC tissue cells was the nucleus, but strong protein expression also led to its localization in the cytoplasm and membrane. Gene Set Enrichment Analysis (GSEA) revealed a relevance between *SNAI1* and *TP53* signaling pathway in LUSC. *SNAI1* can interact with *TP53* and HDAC. By utilizing the CellMiner platform, a wide range of compounds that could potentially target *SNAI1*, including mTOR, were explored. Therefore, potential targeted drugs for *SNAI1* include epigenetic modifications inhibitors and mTOR. Studies have shown that these targeted *SNAI1* agents hold promise for the treatment of LUSC.

Conclusion: High expression of the *SNAI1* gene is significantly associated with poor OS and DFS outcomes in LUSC patients. *SNAI1* serves as an independent prognostic factor for LUSC and can be used as a biomarker for prognostic prediction. *SNAI1* holds promise for the treatment of LUSC.

Keywords: Protein, Zinc, Lung cancer, Zinc finger protein family, Lung squamous cell carcinoma, *SNAI1*, *TP53*

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INTRODUCTION

Lung cancer is one of the most common and deadliest malignant tumours worldwide. The exact mechanisms underlying its development remain unclear and long-term survival rates are low. According to global cancer reports published annually, lung cancer ranks first in both incidence and mortality among malignant tumours. In China, lung cancer also holds the top position in terms of incidence and cause of death, posing a significant threat to the health of our population. The burden of this disease is substantial, making its prevention and control efforts highly challenging.

Squamous cell carcinoma, adenocarcinoma, small cell carcinoma and large cell carcinoma are the most frequent pathological types of lung cancer, accounting for over 90% of cases [1-4]. However, despite their prevalence, the precise mechanisms driving lung carcinogenesis remain poorly understood. Current treatment options

yield unsatisfactory outcomes with low long-term survival rates.

Snail Family Transcriptional Repressor 1 (*SNAI1*) belongs to the zinc finger protein family and plays diverse roles in tumour initiation, progression, drug resistance and stemness properties [5-10]. Previous studies conducted by our research group have highlighted the significant biological functions of *SNAI1* in normal and tumour tissues. Therefore, this study aims to explore the role of *SNAI1* and its potential molecular mechanisms in LUSC.

MATERIALS AND METHODS

NIH/NCBI database analysis

We analyzed the expression of *SNAI1* in normal lung tissue by accessing the databases provided by the National Institutes of Health (NIH) and the National Center for Biotechnology Information (NCBI) through their website (https://www.ncbi.nlm.nih.gov/gene/6615).

GEPIA2 database analysis

We utilized the GEPIA2 database (http://gepia2.cancer-pku.cn), which is a comprehensive resource for analyzing gene expression patterns. The database includes a total of 9,736 tumour samples and 8,587 normal tissue samples from both The Cancer Genome Atlas (TCGA) project and Genotype-Tissue Expression (GTEx) project. In the functional module, we used single-gene analysis to investigate the expression of *SNAI1* in various cancers as well as normal tissues, examining its relationship with clinical staging, Overall Survival (OS) and Disease-Free Survival (DFS). Default thresholds were chosen as reference values.

TCGA database

We downloaded gene expression data and clinical data for LUSC from The Cancer Genome Atlas (TCGA) database and merged them using R-4.2.2 software.

The differentially expressed genes between LUSC patients and healthy controls were analyzed using the LIMMA package, by setting the threshold to greater than 2-fold and an adjusted p-value less than 0.01.

The differentially expressed genes between the high and low expression of *SNAI1* in LUSC patients were analyzed using the LIMMA package, by setting the threshold to greater than 2-fold and an adjusted p-value less than 0.01.

The human protein atlas analysis

SNAI1 protein expression in patients with lung squamous cell carcinoma: The human protein atlas database (https://www.proteinatlas.org/) serves as a valuable resource for SNAI1 protein expression patterns in various tissues and tumour samples. By analyzing the immunohistochemistry data provided by this database, the expression profile of SNAI1 protein specifically in patients with LUSC were examined.

The subcellular localization patterns of SNAI1 protein in LUSC tissues were analyzed through the provides comprehensive immunohistochemistry data on the human protein atlas database.

X-tile software analysis

We performed optimal cut-off value analysis on *SNAI1* gene expression in LUSC patients using X-tile software to determine prognostic stratification levels. The expression levels of SNAI1 gene were categorized into high, medium and low groups and their correlation with survival rates was explored using Kaplan-Meier survival curves.

String protein-protein

Interaction analysis to further investigate the protein-protein interaction network of SNAI1, we utilized the information provided by the string database (https://string-db.org/).

Gene Set Enrichment Analysis (GSEA)

Using GSEA 4.0.3 software, we performed gene set enrichment analysis on gene expression data from LUSC samples in the TCGA database.

Screening potential molecular targeted drugs for SNAI1 using CellMiner

By querying the database with *SNAI1* as the target gene, a list of compounds that have shown significant efficacy against cell lines exhibiting high expression levels of *SNAI1*. These compounds may represent potential candidates for targeted therapy aimed at inhibiting or modulating the function of *SNAI1* in cancer cells.

Statistical analysis

Statistical analyses were performed using IBM SPSS 26.0 Software. The Mann-Whitney U test was used to compare numerical data, while the chi-square test was employed for comparing categorical data. Survival analysis was conducted using the Kaplan-Meier method along with the log-rank test. Univariate and multivariate Cox proportional hazard models were constructed to assess Overall Survival (OS) using a limited backward elimination procedure. The Nomogram plot was build for

predicting the Overall Survival (OS) of cancer patients by R programming language packages such as survival and rms. All statistical tests were two-tailed and a significance level of $p<0.05$ was considered statistically significant.

RESULTS

SNAI1 is highly expressed in normal lung tissue

Transcriptional analysis was performed using high-throughput sequencing of RNA samples from 16 different normal human tissues, including both individual and pooled samples (utilizing the Illumina bodyMap2 transcriptome) within the NCBI database under the biological project PRJEB2445, it was found that in normal human tissues, the expression level of the *SNAI1* gene is approximately 7.0 RPKM (Reads Per Kilobase Million). Importantly, among these normal human tissues, the expression of the *SNAI1* gene in lung tissue exhibited a significantly higher level compared to other tissues. These findings suggest that the *SNAI1* gene plays an important and specific role in normal lung tissue cells at a molecular level (Figure 1A). Data analysis using GEPIA2 also reveals that *SNAI1* exhibits the highest expression level in lung tissues among normal tissues (Figure 1B). This suggests that *SNAI1* plays an important role in the physiological functions of the lungs.

Downregulation of SNAI1 expression in LUSC

To investigate the expression of *SNAI1* in both normal and tumour tissues, analysis was conducted on LUSC data from the TCGA database. Relative to normal control tissues, abnormal expression of *SNAI1* was observed across various tumours. Notably, significant downregulation of *SNAI1* was observed in both LUSC and lung adenocarcinoma (Figures 1B and 1C).

The relationship between *SNAI1* expression and different clinical stages was also examined. Analysis of *SNAI1* expression levels across different clinical stages of LUSC indicated that there were no statistically significant differences at a systems level; however, it was found that the expression levels increased with higher clinical stage progression (Figure 1D).

SNAI1 is significantly associated with OS and DFS in LUSC

Analysis of data from the TCGA database on LUSC, as well as clinical data, revealed a significant correlation between high expression of the *SNAI1* gene and poor Overall Survival (OS) and Disease-Free Survival (DFS) in patients (Figures 2A-2C). The analysis demonstrated that patients with high expression levels of the *SNAI1* gene exhibited lower OS rates compared to those with low expression levels. Similarly, these patients also experienced shorter DFS times, indicating an unfavorable prognosis associated with increased *SNAI1* gene expression. These findings underscore the potential prognostic value of evaluating *SNAI1* expression levels in LUSC patients. High expression of the *SNAI1* gene appears to be indicative of a more aggressive disease phenotype and may serve as a useful biomarker for predicting patient outcomes.

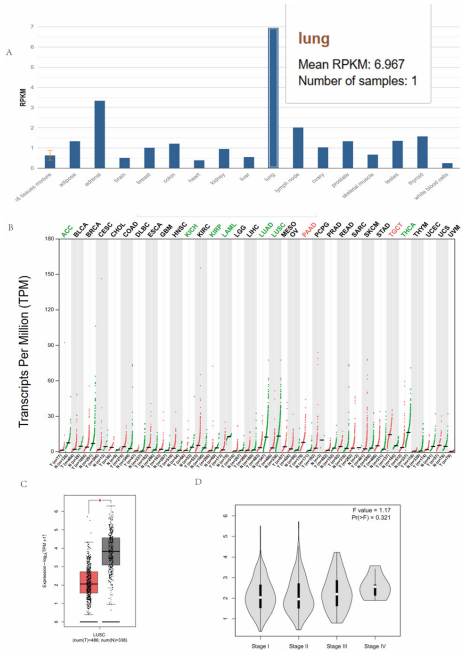

Figure 1. Expression of *SNAI1* in normal and tumor tissues. **Note:** A) Expression levels of the *SNAI1* gene across various types of normal human organs along with their respective standard deviations; B) Expression of *SNAI1* in normal and tumor tissues; C,D) Expression of *SNAI1* in lung squamous cell carcinoma and its expression across different stages.

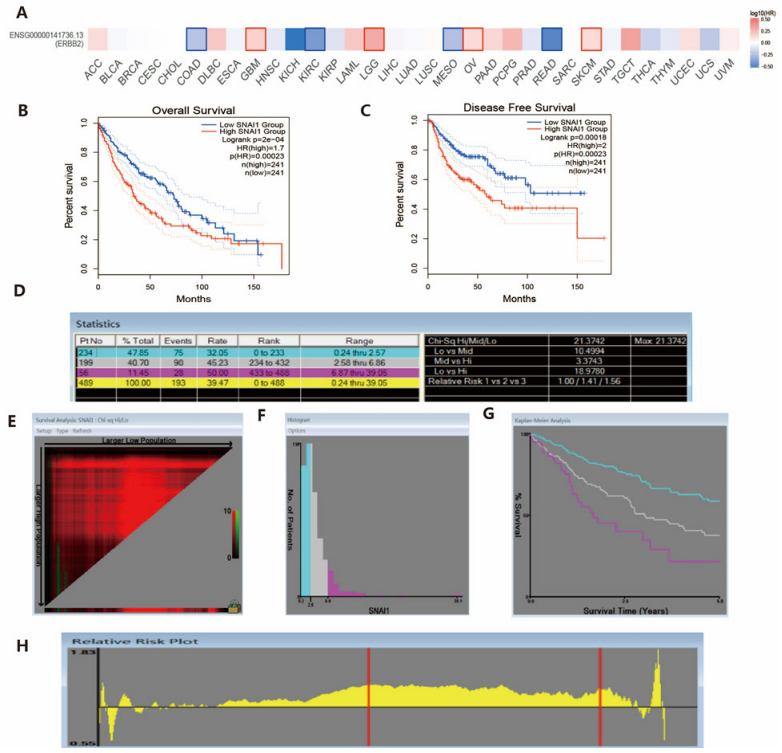

Figure 2. Association of *SNAI1* gene expression with survival prognosis in lung squamous cell carcinoma. **Note:** A) Significant correlation of *SNAI1* with survival in various other tumors; B,C) Significant correlation of *SNAI1* with OS and DFS in lung squamous cell carcinoma, ( ): Low *SNAI1* group; ( ): High *SNAI1* group; D-H) The correlation between different expression groups of *SNAI1* and patient survival prognosis in lung squamous cell carcinoma through X-tile software analysis.

Association of SNAI1 mRNA expression with survival prognosis in LUSC

To analyze the association between *SNAI1* mRNA expression and survival prognosis in LUSC, we merged the expression data of the *SNAI1* gene from TCGA database with clinical data using R programming software. The merged dataset was then subjected to survival analysis using X-tile software. The results revealed that the expression levels of the *SNAI1* gene could be divided into three groups: High, medium and low. The optimal cut-off values for each group were determined to be 2.57 and 6.86. Accordingly, based on these cut-off values, the *SNAI1* gene expression was categorized into high, medium and low three groups. Further analysis was conducted using Kaplan-Meier survival curves to assess the correlation between different expression groups of *SNAI1* and patient survival prognosis in LUSC. These groups exhibited significant differences in their survival curves. The 5-year survival rates were 20.6%, 37.3% and 58% respectively. The median survival times for these groups were 1.7 years, 3.1 years and not reached. The results demonstrated a significant association between high/medium/low expression groups of the *SNAI1* gene and survival outcomes (Figures 2D-2H). This suggests that variations in *SNAI1* gene expression can serve as potential prognostic indicators for patients with LUSC.

SNAI1 is also significantly associated with poor survival in various other tumours

Through the analysis of data from the TCGA database and clinical information pertaining to multiple tumour types, it was discovered that, apart from LUSC, high expression of the *SNAI1* gene is significantly correlated with low Overall Survival (OS) and Disease-Free Survival (DFS) rates in several other malignancies. These include lung adenocarcinoma, colon adenocarcinoma, esophageal cancer, low-grade glioma of the brain, ovarian serous cystadenocarcinoma, gastric adenocarcinoma, thyroid cancer, pleomorphic glioblastomas multiforme as well as renal papillary cell carcinoma. These findings suggest that increased expression of *SNAI1* gene contributes to an unfavorable prognosis for patients across a diverse range of malignant tumours. The observed correlation between *SNAI1* expression and OS/DFS highlights its potential role as a prognostic biomarker in various pathological subtypes such as squamous cell carcinoma, adenocarcinomas (lung adenocarcinoma, colon adenocarcinoma), neuroepithelial neoplasms (low-grade glioma, biphasic glioblastomas) and other tumour types (renal papillary cell carcinoma, malignant mesotheliomas) (Figure 3).

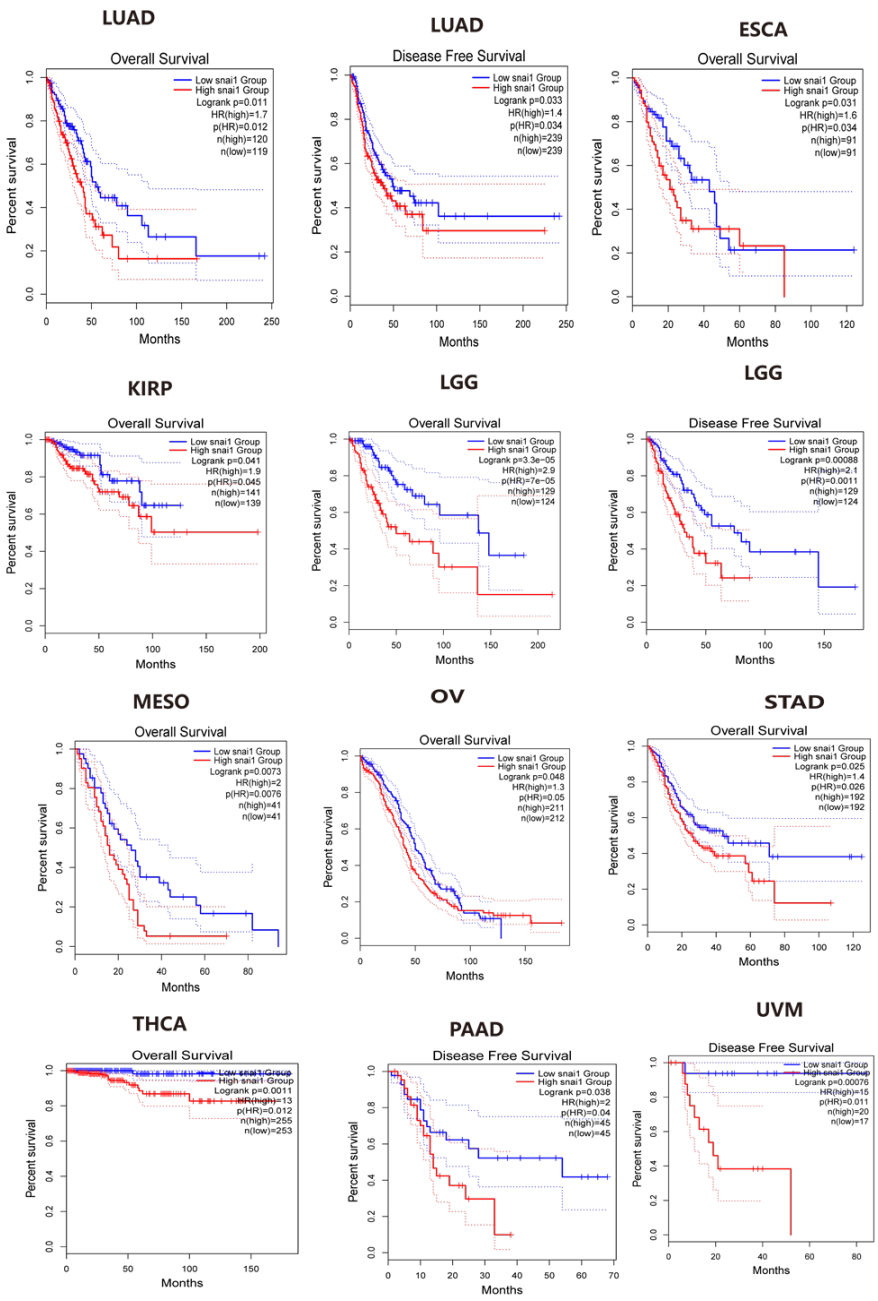

Figure 3. Significant correlation of *SNAI1* with survival in various other tumors. **Note:** LUAD: Lung adenocarcinoma; ESCA: Esophageal Carcinoma; KIRP: Kidney Renal Papillary cell carcinoma; LG: Brain Lower Grade Glioma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; STAD: Stomach Adenocarcinoma; THCA: Thyroid Carcinoma; PAAD: Pancreatic Adenocarcinoma; UVM: Uveal Melanoma; ( ): Low *SNAI1* group; ( ): High *SNAI1* group.

Relationship between expression of SNAI1 gene and clinical features

The expression levels of *SNAI1* were categorized into high and low groups and the statistical significance of various clinical features between these two groups was compared. High *SNAI1* expression in LUSC showed a significant correlation with age, stage, T, N, M and survival time (days), survival status ($p<0.05$) (Table 1). Our findings suggest a significant correlation between *SNAI1* expression and the expression of *SNAI2* and *SNAI3*, indicating a potential relationship among members of the SNAI family. However, no significant correlation was observed between the expression levels of *SNAI2* and *SNAI3* and the prognosis of lung cancer patients (data not shown). Univariate and multivariate Cox regression analysis demonstrated that *SNAI1* expression, age, stage, T, N, M were significantly associated with clinical prognosis. Therefore, the findings suggest that *SNAI1* is an independent prognostic factor for patients with LUSC (Tables 2 and 3).

Table 1. Clinical characteristics of patients in different *SNAI1* mRNA expression groups.

Clinical features		**N**	***SNAI1* mRNA**				**p-value**
			High		**Low**		
			N	**%**	**N**	**%**	
Gender	Male	492	180	73%	184	75%	0.757
	Female		66	27%	62	25%	
Age		492	69	63, 74	67	60, 73	0.03
	≤ 60		30	12%	51	21%	0.041
	>60		216	88%	195	79%	
T	T1	492	56	23%	54	22%	0.025
	T2		117	48%	152	62%	
	T3		51	21%	29	12%	
	T4		22	9%	11	4%	
Lymph node metastasis	N0	492	128	52%	156	63%	0.043
	N1		72	298%	65	26%	
	N2		34	14%	21	8.50%	
	N3		11	4%	4	1.60%	
Distant metastasis	M0	492	201	82%	244	99%	<0.001
	M1		45	18%	2	0.80%	
Stage	I	492	125	51%	117	48%	0.036
	II		68	28%	91	37%	
	III		47	19%	36	15%	
	IV		6	2.40%	2	0.80%	
SNAI1		492	4.56	3.51, 6.72	1.62	1.14, 2.10	<0.001
SNAI2		492	21	12, 30	22	16, 31	0.04
SNAI3		492	0.89	0.59, 1.36	0.55	0.36, 0.91	<0.001
Survival time days		492	470	211, 855	661	358, 1233	<0.001
Survival status	Alive	492	133	54%	164	67%	0.004
	Dead		113	46%	82	33%	

Table 2. Univariate analysis for OS.

	HR	**95% CI**	**p-value**
SNAI1	1.046	1.014-1.078	0.004
Age	1.03	1.004-1.056	0.022
Sex	1.102	0.791-1.536	0.567
Clinical stage	1.269	1.07-1.504	0.006
T stage	1.37	1.141-1.645	0.001
Lymph node metastasis	1.213	1.008-1.473	0.048
Distant metastasis	1.852	1.13-3.034	0.014

Table 3. Multivariate analysis for OS.

	HR	**95% CI**	**p-value**
SNAI1	1.044	1.006-1.083	0.023
Age	1.019	1.001-1.038	0.036
Clinical stage	1.083	1.053-1.227	0.045
T stage	1.369	1.058-1.771	0.017
Lymph node metastasis	1.325	1.074-1.802	0.034
Distant metastasis	1.684	1.0096-2.807	0.046

Construction of a nomogram to predict the survival of patients with LUSC in order to help clinical diagnosis and treatment

To predict the survival outcomes of LUSC patients, a nomogram was constructed based on the results of multivariable Cox regression analysis. Statistical significant variables were included in the construction of the nomogram, which serves as a graphical prediction model for LUSC patients. The nomogram provides scores corresponding to different clinical and pathological characteristics of the patients. By summing up these scores, we can obtain a total score that predicts the Overall Survival (OS) of lung cancer patients at 1 and 2 years.

The construction of this prediction model is based on the results of multivariable Cox regression analysis, which takes into account the influence of multiple variables and determines their independent contributions to survival rates. By incorporating these statistically significant variables into the model, we can more accurately predict the survival outcomes of LUSC patients (Figure 4).

High expression of SNAI1 protein in LUSC is associated with poor prognosis

We have discovered a significant correlation between the levels of *SNAI1* gene mRNA and the clinical prognosis of patients. To investigate the relationship between the expressions levels of SNAI1 protein and prognosis in patients with LUSC, the human protein atlas database for analysis. The research results demonstrate that high expression of SNAI1 protein in lung cancer is associated with poor prognosis. Specifically, the 5-year survival rate for patients in the high expression group of SNAI1 protein was 37%, while it was 59% for patients in the low expression group ($p<0.05$), indicating a significant statistical difference between the two groups. These findings conclude that high expression of SNAI1 protein is associated with poor prognosis in patients with LUSC (Figure 5A).

The primarily subcellular localization of the SNAI1 protein in LUSC tissue cells is the cell nucleus

To investigate the subcellular localization of the SNAI1 protein in LUSC tissue cells, the immunohistochemistry data from the human protein atlas database was utilized to analyze the expression of SNAI1 protein in patients with LUSC. The results found that regardless of low, moderate, or high expression levels, the SNAI1 protein is primarily localized in the cell nucleus. However, in cases of strong expression, cytoplasmic and membrane localization of the protein was also observed. Our findings demonstrate that in lung cancer tissue, the SNAI1 protein is predominantly localized in the cell nucleus, irrespective of its expression level. This nuclear localization suggests the importance of *SNAI1* in gene transcription and regulation. However, it is worth noting that in cases of strong protein expression, we also observed cytoplasmic and membrane localization. This subcellular localization variation may be related to other functions or signaling pathways of *SNAI1* in lung cancer (Figure 5B).

Biological mechanisms of SNAI1 in LUSC and its association with the P53 signaling pathway

To investigate the molecular mechanisms of *SNAI1*, we conducted an analysis using gene set enrichment analysis to examine the relationship between *SNAI1* expression and hallmark gene sets as well as oncogenic signature gene sets. Interestingly, our findings revealed a significant association between *SNAI1* and the *TP53* signaling pathway in both gene sets (Tables 4 and 5, Figures 6A-6C).

Interaction between SNAI1 and TP53, epigenetic modification proteins

Protein-protein interaction analysis of *SNAI1* was conducted using the online tool string 10.0 from the Protein-protein interaction database (http://www.string-db.org/). The analysis revealed several proteins that interact with *SNAI1*, including *TP53*, epigenetic modification (*EZH2*, *HDAC1*, *HDAC2*, *KDM1A*), *JUN*, *MTA3* (Figures 6A-6D).

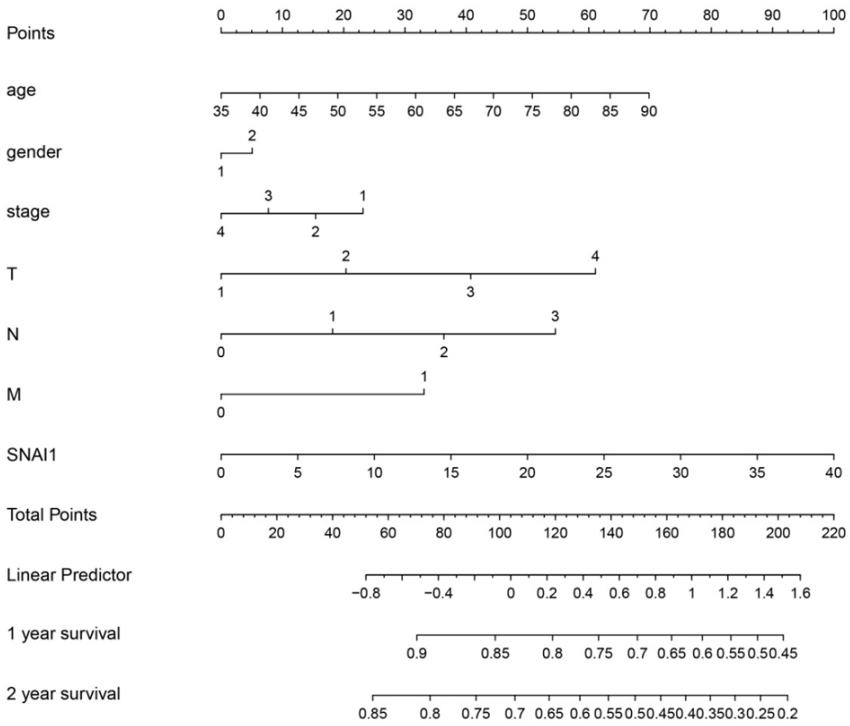

Figure 4. Construction of a nomogram to predict the survival of patients with LUSC.

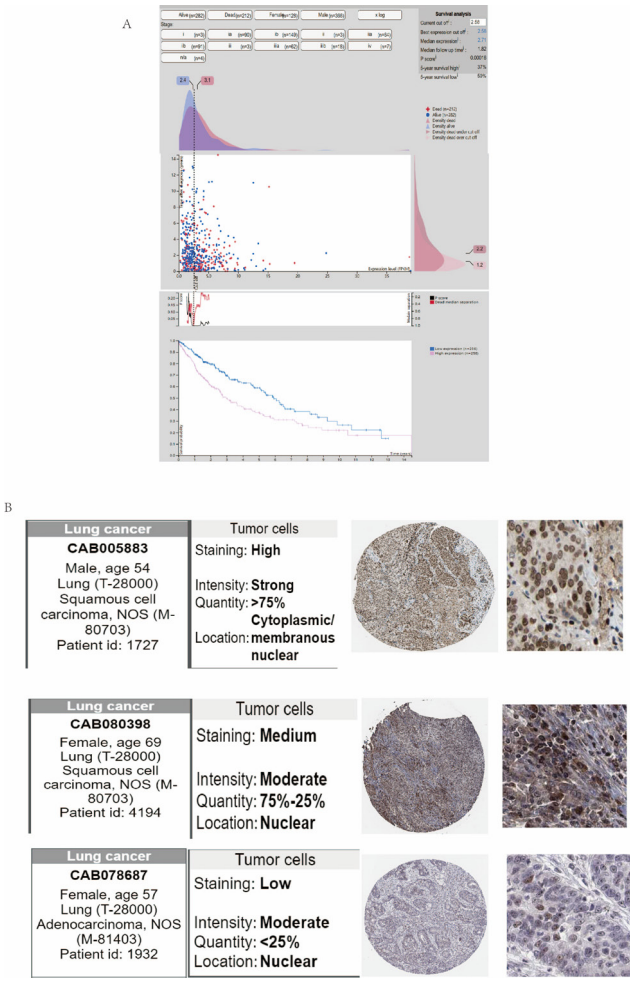

Figure 5. The expression of SNAI1 protein in LUSC. **Note:** A) High expression of SNAI1 protein in LUSC is associated with poor prognosis; B) The subcellular localization of the SNAI1 protein in LUSC tissue cells.

Table 4. The hallmark gene sets correlated with *SNAI1* expression.

Name	ES	NES	p-value	FDR q-val
HALLMARK_TNFA_SIGNALING_*VIA*_NFKB	0.44	2.09	0	0
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.42	2.06	0	0.001
HALLMARK_P53_PATHWAY	0.43	2.06	0	0.001
HALLMARK_HYPOXIA	0.4	1.94	0	0.001
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.47	1.81	0.002	0.002
HALLMARK_UV_RESPONSE_UP	0.35	1.65	0	0.013
HALLMARK_INFLAMMATORY_RESPONSE	0.33	1.63	0	0.013
HALLMARK_GLYCOLYSIS	0.31	1.48	0.005	0.047
HALLMARK_INTERFERON_GAMMA_RESPONSE	0.28	1.38	0.01	0.113
HALLMARK_APICAL_JUNCTION	0.28	1.35	0.027	0.121
HALLMARK_ADIPOGENESIS	0.28	1.35	0.023	0.113
HALLMARK_COMPLEMENT	0.28	1.34	0.025	0.107

Table 5. The correlation between *SNAI1* expression and oncogenic signature gene sets.

Name	ES	NES	p-value	FDR q-val
TGFB_UP.V1_UP	0.44	2.11	0	0
PRC2_*EZH2*_UP.V1_UP	0.41	1.99	0	0.001
MEL18_DN.V1_UP	0.4	1.83	0	0.006
RELA_DN.V1_UP	0.39	1.82	0	0.005
BMI1_DN_MEL18_DN.V1_UP	0.39	1.79	0	0.005
P53_DN.V1_DN	0.34	1.65	0	0.026
STK33_NOMO_UP	0.31	1.59	0	0.046
CAHOY_ASTROGLIAL	0.37	1.57	0.007	0.048
ESC_V6.5_UP_EARLY.V1_DN	0.33	1.54	0.002	0.063
IL2_UP.V1_UP	0.32	1.51	0	0.08
VEGF_A_UP.V1_UP	0.31	1.5	0.002	0.081

The role of the SNAI1-related TP53 signaling pathway gene set in LUSC

GSEA analysis revealed a correlation between the expression of *SNAI1* and the abnormal expression of key genes in the *TP53* signaling pathway. To investigate the role of the *SNAI1*-related *TP53* signaling pathway gene set in lung cancer, the intersecting gene sets were obtained by comparing the expression of the gene sets related to the *TP53* signaling pathway, the differentially expressed genes in LUSC patients and normal healthy controls, as well as the differentially expressed gene sets based on the high and low expression of *SNAI1* (Figure 6E).

The total number of differentially expressed genes between LUSC patients and healthy controls was 5948. There were 4005 genes differentially expressed based on the high and low expression of *SNAI1*. The intersection of these three sets of differentially expressed genes revealed 15 common genes: *ABCC5, AK1, ATF3, FAS, PPP1R15A, NDRG1, SLC19A2, STOM, ST14, FDXR, TGFA, RRAD, PLK3, JUN, TXNIP.*

Prognostic analysis was performed to assess the relationship between these 15 genes and the prognosis of LUSC patients. It was found that the expression of these 15 genes was associated with poor prognosis in LUSC. This suggests that the *SNAI1*-related *TP53* signaling pathway gene set in LUSC is associated with poor prognosis in patients with LUSC (Figure 6F).

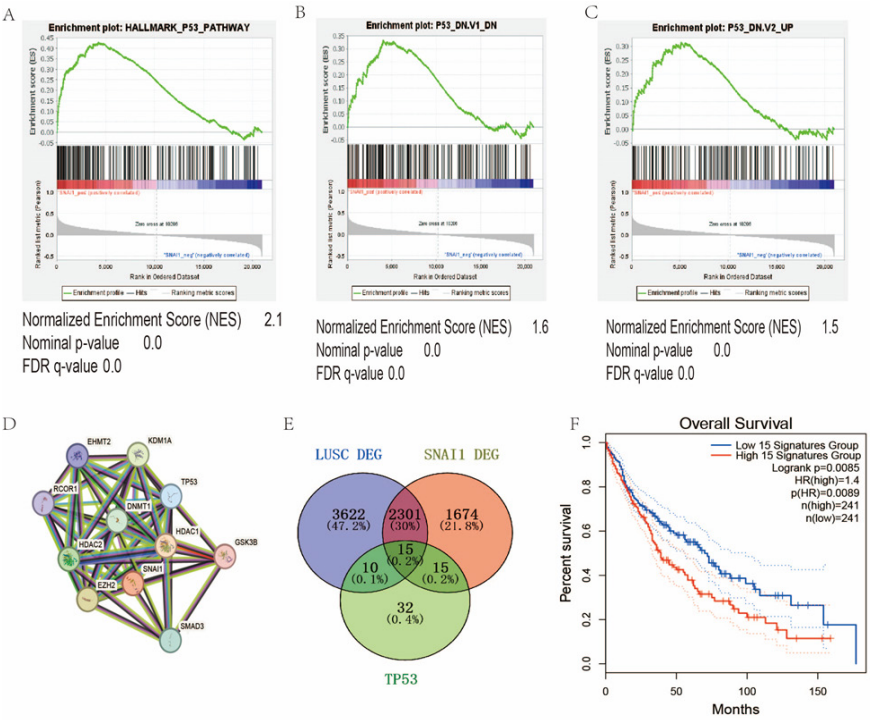

Figure 6. Biological mechanisms of SNAI1 in LUSC. **Note:** A-C) The molecular mechanisms of SNAI1 were associated with *TP53* signaling pathway by gene set enrichment analysis; D) Interacting proteins of SNAI1; E) The intersection of three sets of differentially expressed genes: The differentially expressed genes between LUSC patients and healthy controls, the differentially expressed genes differentially expressed based on the high and low expression of SNAI1, SNAI1-related *TP53* signaling pathway genes; F) The 15 SNAI1-related *TP53* signaling pathway genes was associated with poor prognosis in LUSC; ( ): Low 15 signatures group; ( ): High 15 signatures group.

Identification of potential molecular targeted drugs for SNAI1

By utilizing the CellMiner platform, we were able to explore a wide range of compounds that could potentially target *SNAI1*, including mTOR (Supplementary Table 1). The mechanistic Target of Rapamycin (mTOR) is a central signaling pathway that plays a crucial role in regulating cell proliferation and survival. In the context of lung cancer, it has been reported that mTOR is frequently overexpressed. Therefore, targeting the mTOR pathway for down-regulation has emerged as a promising strategy in lung cancer therapy [11-15].

Protein-protein interactions have revealed that *TP53*, *Histone Deacetylase (HDAC)*, *HDAC1* and *HDAC2* interact with *SNAI1*, suggesting that they could be potential target molecules of *SNAI1*. Among these compounds, HDAC inhibitors emerged as promising candidates. HDAC inhibitors are known to modulate gene expression by altering chromatin structure and function, which may play a crucial role in regulating *SNAI1* activity. Numerous preclinical and clinical studies have demonstrated that HDAC inhibitors can be used alone or in combination for the treatment of lung cancer, showing remarkable therapeutic efficacy. Many ongoing clinical trials are further evaluating the profound impact of HDAC inhibitors on the treatment outcomes of lung cancer (Supplementary Tables 2 and 3).

DISCUSSION

This study found that *SNAI1* is most abundantly expressed in lung tissue compared to other organs. This finding suggests a potential role for *SNAI1* in the regulation and functioning of lung tissue. However, the exact function of *SNAI1* in normal lung tissue cells remains unclear. The expression of *SNAI1* was significantly downregulated in LUSC. Abnormal expression of *SNAI1* has been observed in various tumours compared to normal control tissues. Both LUSC and adenocarcinoma showed significant downregulation of *SNAI1* expression.

High expression of the *SNAI1* mRNA is significantly associated with poor Overall Survival (OS) and Disease-Free Survival (DFS) in patients with LUSC, indicating its negative prognostic impact. Similar associations between high *SNAI1* gene expression and poor OS/DFS have been observed in various other tumours. Stratified analysis using X-tile software on *SNAI1* gene expression revealed distinct survival outcomes among groups with high, medium, or low expressions of *SNAI1*.

The *SNAI1* mRNA expression was significantly correlated with age and distant metastasis. Univariate Cox regression

analysis found that *SNAI1* expression along with age, T stage, lymph node metastasis, distant metastasis were all significantly associated with clinical prognosis. Multivariate Cox regression analysis further confirmed that *SNAI1* expression along with age, T stage, lymph node metastasis, distant metastasis were independent prognostic factors.

The high expression of SNAI1 protein in lung cancer is also associated with poor prognosis. The 5-year survival rate for patients in the high expression group of SNAI1 protein was 37%, compared to 59% for patients in the low expression group. These research findings conclude that the high expression of SNAI1 protein is associated with poor prognosis in patients with LUSC. The primarily subcellular localization of the SNAI1 protein in LUSC tissue cells is the cell nucleus, but strong protein expression also leads to cytoplasmic and membrane localization of *SNAI1*. This variation in subcellular localization may be associated with other functions or signaling pathways of *SNAI1* in lung cancer. To better understand this phenomenon, further research is needed on the functions and regulatory mechanisms of the SNAI1 protein. Firstly, we can consider the role of *SNAI1* in the cell nucleus. As a transcription factor, *SNAI1* regulates gene transcription in the cell nucleus and is involved in the process of Epithelial-Mesenchymal Transition (EMT), which is closely associated with cell morphological changes and tumour invasion and metastasis. However, the cytoplasmic and membrane localization of *SNAI1* suggests that this protein may also play a role in other cellular functions and signaling pathways. The cytoplasmic localization may be related to the degradation, post-translational modifications, and intercellular signaling of *SNAI1*. The membrane localization may involve the regulation of *SNAI1* in interaction with other cell surface receptors or intercellular interactions. Furthermore, the variation in subcellular localization of *SNAI1* may also be associated with the development and prognosis of LUSC. So, the variation in subcellular localization of the SNAI1 protein in LUSC may be associated with its multiple functions and signaling pathways involved in tumour development, invasion and metastasis. Further research on the localization mechanisms and functional regulation of *SNAI1* will contribute to a better understanding of the occurrence and progression of LUSC.

Gene Set Enrichment Analysis (GSEA) revealed relevance between *SNAI1* and *TP53* signaling pathway in LUSC. *SNAI1* can interact with *TP53*, key regulators of epigenetic proteins (*EZH2, HDAC1, HDAC2, KDMIA*) to exert biological functions. The *TP53* signaling pathway is one of the main cellular apoptosis signaling pathways that plays a crucial role in various tumours and is known as the guardian of life [16-25].

CONCLUSION

The interaction between *SNAI1* and *TP53* may contribute to the aggressive behaviour of cancer cells. Furthermore, *SNAI1*'s interactions with *HDAC1* and *HDAC2* suggest its involvement in chromatin re-modelling and transcriptional regulation. The recruitment of histone deacetylases by *SNAI1* can lead to epigenetic changes that promote tumour cell survival and metastasis. Therefore, potential targeted drugs for *SNAI1* include epigenetic modifications inhibitors and mTOR. Studies have shown that these targeted *SNAI1* agents hold promise for the treatment of lung cancer.

AVAILABILITY OF DATA AND MATERIALS

The data and materials are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare no competing interests.

FUNDING

This study was supported by the Construction Project KHGMY1357647 from the Medical Science and Technology Research of Celement Project and Health Commission.

AUTHORS' CONTRIBUTIONS

(I) Conception and design: Caren and Maigna; (II) Administrative support: Caren; (III) Data analysis and interpretation: Caren; (IV) Manuscript writing: Caren; (V) Final approval of manuscript: All authors.

ACKNOWLEDGEMENTS

We thank the editor and reviewers for relevant and helpful comments on the manuscript.

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