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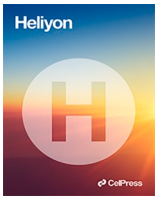

Research article

Bioinformatics analysis and validation of CSRNP1 as a key prognostic gene in non-small cell lung cancer

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ABSTRACT

Cysteine and serine-rich nuclear protein 1 (CSRNP1) has shown prognostic significance in various cancers, but its role in non-small cell lung cancer (NSCLC) remains elusive. We investigated CSRNP1 expression in NSCLC cases using bioinformatics tools from the GEO public repository and validated our findings through RT-qPCR in tumor and adjacent normal tissues. KEGG and GO enrichment analyses were employed to unveil the significant deregulation in signaling pathways. Additionally, clinical significance of CSRNP1 in NSCLC was determined through receiver operating curve (ROC) analysis, and its impact on survival was assessed using Kaplan-Meier analysis. To explore the functional impact of CSRNP1, we silenced its expression in NSCLC cells and assessed the effects on cell viability, migration, and invasion using MTT, Transwell, and wound-healing assays, respectively. Additionally, we investigated the influence of CSRNP1 silencing on the phosphorylation patterns of critical signaling proteins such as p53, *p*-Akt, and *p*-MDM2. Our results demonstrated significantly lower CSRNP1 expression in NSCLC tumor tissues ($P < 0.01$). ROC analysis indicated that NSCLC patients with high CSRNP1 expression exhibited extended overall survival and disease-free survival. Furthermore, CSRNP1 silencing promoted NSCLC cells viability, migration, and invasion ($P < 0.05$). Mechanistically, CSRNP1 silencing led to increased phosphorylation of AKT and MDM2, along with a concurrent reduction in p53 protein expression, suggesting its impact on NSCLC through deregulated cell cycle processes. In conclusion, our study underscores the significance of CSRNP1 in NSCLC pathogenesis, offering insights for targeted therapeutic interventions of NSCLC.

1. Introduction

World health organization classified Non-small cell lung cancer (NSCLC) into three main types including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [1]. This categorization is pivotal for understanding the histological diversity of NSCLC, which collectively constitutes nearly 85% of all lung cancer cases [2–5]. The clinical presentation of NSCLC often involves symptoms such as cough, hemoptysis, dyspepsia, and chest discomfort [6]. In the past few years, there have been notable progressions in discovering prognostic and diagnostic biomarkers for NSCLC. Additionally, research has been conducted on diverse therapeutic

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strategies, such as targeted therapy, chemotherapy, immunotherapy, and radiotherapy [7,8]. Nevertheless, the prognosis for advanced NSCLC patients remains bleak, as the disease is often diagnosed at advanced stages (III or IV according to AJCC staging), rendering radical treatment unattainable in approximate 75% of cases [9]. Furthermore, despite the availability of these therapies, drug resistance is an inevitable challenge [10–14], and pursuit of novel therapeutic targets, identification of new targeted genes and pathways, development of effective therapeutics, and discovery of biomarkers are crucial priorities for advancing the comprehensive treatment of NSCLC.

The cysteine-serine-rich-nuclear protein (CSRNP) family, comprising CSRNP1, CSRNP2, and CSRNP3, holds notable biological significance [15,16]. These nuclear proteins, along with their associated transcription factors, demonstrate evolutionary conservation [17]. Specifically, DNA methylation at key sites, such as Cg19538674 for CSRNP1, Cg07772537 for CSRNP2, and Cg07811002 for CSRNP3, emerges as a critical factor impacting the overall survival of patients with clear cell renal cell carcinoma [15]. Gene expression analysis from GEO datasets highlights aberrant expression of CSRNP2 in obese individuals compared to controls [18], while CSRNP3 exhibits low methylation levels in lung tissues [19].

CSRNP1, characterized by a 3.2 kb transcript, demonstrates ubiquitous expression across human tissues, with lung exhibiting the highest abundance [20]. Its potential role as a tumor suppressor is underscored by frequent downregulation in various cancers, including lung cancer [20]. CSRNP1 has been explored as a prognostic biomarker in diverse cancers, such as prostate, kidney, and liver cancers, emphasizing its pivotal prognostic value [15,21–24]. Notably, CSRNP1 emerges as a gene associated with neutrophil differentiation, proving predictive of total survival time in NSCLC patients [25] and has been identified as a prognostic biomarker in lung squamous cell carcinoma patients [26]. While CSRNP1's impact on neural progenitor cell proliferation and survival reduction is established [27], its inhibition suppresses apoptosis in cementoblasts [28], and it exhibits a pro-apoptotic effect in cardiomyocytes [29]. Intriguingly, the role of CSRNP1 in NSCLC remained unexplored. Leveraging integrative bioinformatics analysis, we sought to unravel the potential implications of CSRNP1 in NSCLC. Concurrently, we conducted knockdown experiments to elucidate the functional role of CSRNP1 in NSCLC cells.

The main goal of this study was to clarify the biological mechanisms involving CSRNP1 in NSCLC. Our investigation aimed to address a significant gap in our understanding of the role of CSRNP1 in NSCLC. Through this research, we aim to advance our knowledge of NSCLC pathogenesis, ultimately contributing to improving patient outcomes.

2. Materials and methods

2.1. Bioinformatics analysis

We employed the versatile ASSISTANT for Clinical Bioinformatics platform (https://www.aclbi.com/static/index.html#/) and this web-based tool facilitated a comprehensive suite of bioinformatics analyses, encompassing the generation of diverse visual outputs, including box plots, principal component analysis (PCA) biplots, volcano maps, heatmaps, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and Gene Ontology (GO) analysis. Data processing and visualization were executed using R v4.0.3 software, enhancing the interpretability of results. Microarray data from the GSE40275 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40275) were sourced, with raw data acquired in MINiML format. The limma package within the R software facilitated the exploration of differentially expressed mRNA, and careful consideration of adjusted P-values was undertaken to rectify potential false-positive outcomes inherent in GEO datasets. To gain deeper insights into mRNA's role in carcinogenesis, we utilized the ClusterProfiler package (version: 3.18.0) within the R software. This package enabled a comprehensive analysis of Gene Ontology (GO) functions associated with potential targets and KEGG pathways, providing a nuanced understanding of the molecular mechanisms at play. The resulting findings were succinctly presented using the heatmap package, contributing to a visually informative representation of the

Table 1
Clinicopathologic characteristics of 30 NSCLC patients.

Serial number	Characteristics	Number	(%)
1	Gender		
	Female	11	36.7
	Male	19	63.3
2	**Age (years)**		
	>60	17	56.7
	≤60	13	43.3
3	**Histology**		
	Squamous	13	43.3
	Adenocarcinoma	17	56.7
4	**Tumor size**		
	>3 cm	12	40
	≤3 cm	18	60
5	**TNM stage**		
	I-II	22	73.3
	III-IV	8	26.7
6	**Smoking history**		
	yes	20	66.7
	no	10	33.3

intricate relationships within the dataset.

2.2. Patients

Thirty patients with NSCLC were enrolled from June 2019 to August 2021 at the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University. The clinicopathologic characteristics of these NSCLC patients are provided in Table 1.

The study was approved by the Ethics Committee of Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University and conducted in the light of the guidelines of the Declaration of Helsinki. All patients had signed informed consents before study.

2.3. Cell culture and transfection

BEAS-2B (Control), A549, H1650, Calu-1, NCI–H292, and NCI–H23 cell lines, sourced from the American Type Culture Collection (ATCC, USA), were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Gibko, USA), and Penicillin. The cells were grown in a 12-well plate at 37 °C with 5% CO2 and harvested upon reaching 80% confluency. For the specific silencing of CSRNP1, three small interfering RNA (Si)-CSRNP1 plasmids and a Si-negative control (Si-NC) plasmid were obtained from GenePharma (Shanghai, China) and transfected into the cells using Lipofectamine 300 (Invitrogen, USA) for a 48-h period. The RNA oligo sequences for Si-CSRNP1 plasmids and Si-NC plasmid are detailed as follows in Table 2.

2.4. RT-qPCR

Total RNA in the tissues specimens were isolated by TRIzol reagent after transfection. Afterwards, RNA was reverse transcribed to cDNA through the reverse transcript kit (Beyotime Biotechnology, Shanghai, China). Routine reaction steps of PCR were conducted. Each sample was run in triplicate according to the above three steps, and the data were quantitatively analyzed according to the $2^{-\triangle\triangle CT}$ method. The RT primers used were following: CSRNP1 5′-F: AGGCGCCAGAGGCACCATGGAC-3′, R: 5′-CATCCTGTGCGTTGGCTGCC-3′, GAPDH, F: 5′-GAAGGTGAAGGTCGGAGTC-3′, R: 5′-GAAGATGGTGATGGGATTT-3'.

2.5. Microculture Tetrazolium (MTT)

Cells after transfection were cultivated in plates of 96-well (#FCP963, Beyotime, Shanghai, China) at density of 5×10^3 cells/well. 10 μL MTT solution (Beyotime, China) was supplemented into cells for 4 h of incubation. The optical density (OD) value at 570 nm was assessed via a microplate reader (Spectramax 190, Molecular Devices, San Jose, CA, USA).

2.6. Wound scratch assays

Calu-1 and NCI–H292 cells after transfection were plated into the plate of 6 wells and cultivated until reaching 80% confluence. Next, a sterile pipette (200 μL) tip was used for creating scratch. Subsequently, cells were washed to clear off the dislodged cells. After 48 h, the pictures of the wound healing to access the cells migration ability images were acquired using a light microscope (Leica). The wound healing rate is determined by the formula: (wound width at 0 h - wound width at 48 h) divided by the wound width at 0 h.

2.7. Transwell assay

Matrigel was covered on the transwell chamber (Corning, China), and 2×10^4 cells were incubated in serum-free medium in the upper chamber. The bottom chamber was supplemented with 500 μL medium containing 10% FBS. After 48 h, cells remaining in the upper chamber were removed, and cells in the bottom chamber were stained with crystal violet as described previously [30]. Number of stained cells was observed under a light microscope (Leica).

2.8. Western blots

Cells were lysed by RIPA assay buffer (Beyotime, China). Protein was parted by SDS-PAGE, followed by transferring onto PVDF membranes (Millipore, China). The membranes were sealed with skim milk (5%) and incubated with primary antibodies CSRNP1 (LS-C346243, lsbio, USA, 1:1000), *p*-Akt (9271, CST, 1:800), Akt (NB100-56749, Novus Biologicals, 1:300), *p*-MDM2 (3521, CST, 1:1000), MDM2 (ab259265, Abcam, 1:1500), p53 (MA1-12549, ThermoFisher scientific, 1:150), CDK1 (ab245318, Abcam, 1:2000), Cyclin B

Table 2
Sequences of small interfering RNA (Si)-CSRNP1 plasmids and a Si-negative control (Si-NC).

Sequence	SiRNA Type	Guide Strand	Passenger Strand
si-CSRNP1#1	CSRNP1-targeted	5′-AGAAUUGUUUGAAAACCAGGA-3′	5′-CUGGUUUUCAAACAAUUCUCU-3′
si-CSRNP1#2	CSRNP1-targeted	5′-AAACAUUAGCAAACAAAGGGU-3′	5′-CCUUUGUUUGCUAAUGUUUAA-3′
si-CSRNP1#3	CSRNP1-targeted	5′-AUGGAAUAAAUUAUAGUUCUG-3′	5′-GAACUAUAAUUUAUUCCAUUU-3′
si-NC	Negative control	5′-UUCUCCGAACGUGUCACGUTT-3′	5′-ACGUGACACGUUCGGAGAATT-3′

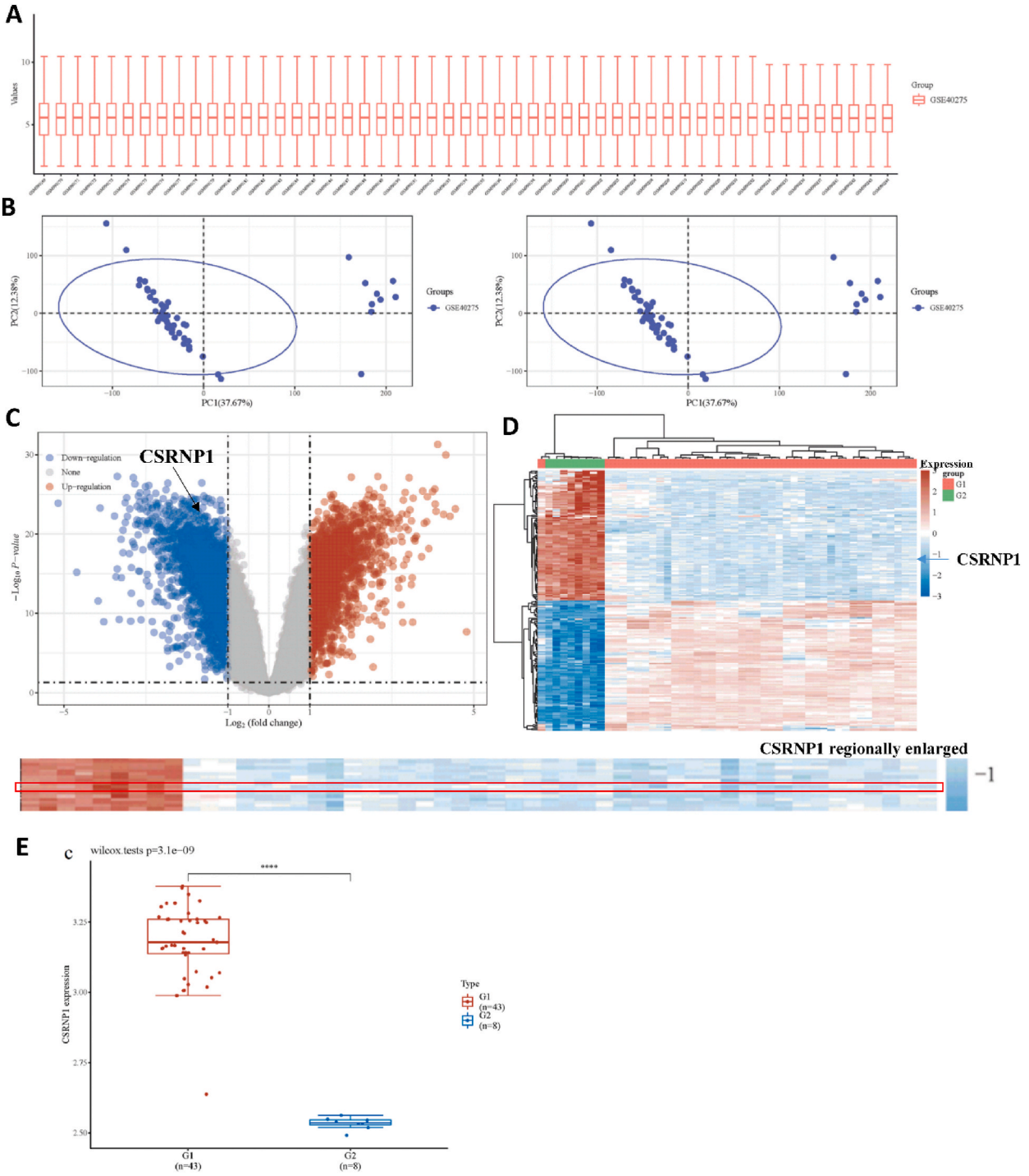

Fig. 1. Screening of the DEGs in NSCLC patients and healthy controls. *(A) Boxplot illustrating the normalized gene expression data from the GEO database (GSE124272). Each row represents a sample, and columns represent the corresponding gene expression values within the samples. (B) PCA showed that DEGs can disting anduish NSCLC patients from healthy controls in* GSE40275 *gene chip. (C) Volcano plot is representing the dysregulated expression of genes including CSRNP1. (D) The heatmap visually represents the differential gene expression between two groups, highlighting both up-regulated (red part) and down-regulated (blue part) genes. Notably, CSRNP1 stands out as a downregulated gene, suggesting its potential role in the observed expression patterns. (E) CSRNP1 expression in two groups of participants using GEO database by box plots. G1: healthy group; G2: NSCLC group. ****p < 0.0001.*

(ab181593, Abcam, 1:2000) and GAPDH (ab181602, Abcam, USA, 1:1500) overnight at 4 °C, followed by treating with HRP-conjugated goat anti-rabbit secondary antibody (ASPEN Biotechnology, #AS1107, 1:1500) and goat anti-mouse secondary antibody (Invitrogen, #62–6520, 1:2000) for 1 h. Furthermore, the brands were examined by an ECL reagent (Thermo Scientific Pierce, USA).

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism software. Data was presented as mean ± standard deviation. The independent two-sample *t*-test was used to compare means between two groups, while one-way analysis of variance (ANOVA) was used to assess the differences among multiple groups. The clinical significance of CSRNP1 expression in peripheral blood samples was evaluated using receiver operating curve (ROC) analysis. ROC curves, generated by plotting true positive rate against false positive rate, elucidate the sensitivity-specificity relationship, providing insights into the diagnostic utility of CSRNP1 expression. Kaplan-Meier survival curves, analyzed with the Mantel-Cox test, depicted CSRNP1 expression-related overall survival (OS) and disease-free survival (DFS). High or low expression levels were determined based on the median expression value of CSRNP1 in NSCLC tissues.

3. Results

3.1. Screening of genes in NSCLC patients and healthy control group

We first aimed to identify differentially expressed genes (DEGs) in individuals with NSCLC compared to a normal control group. We

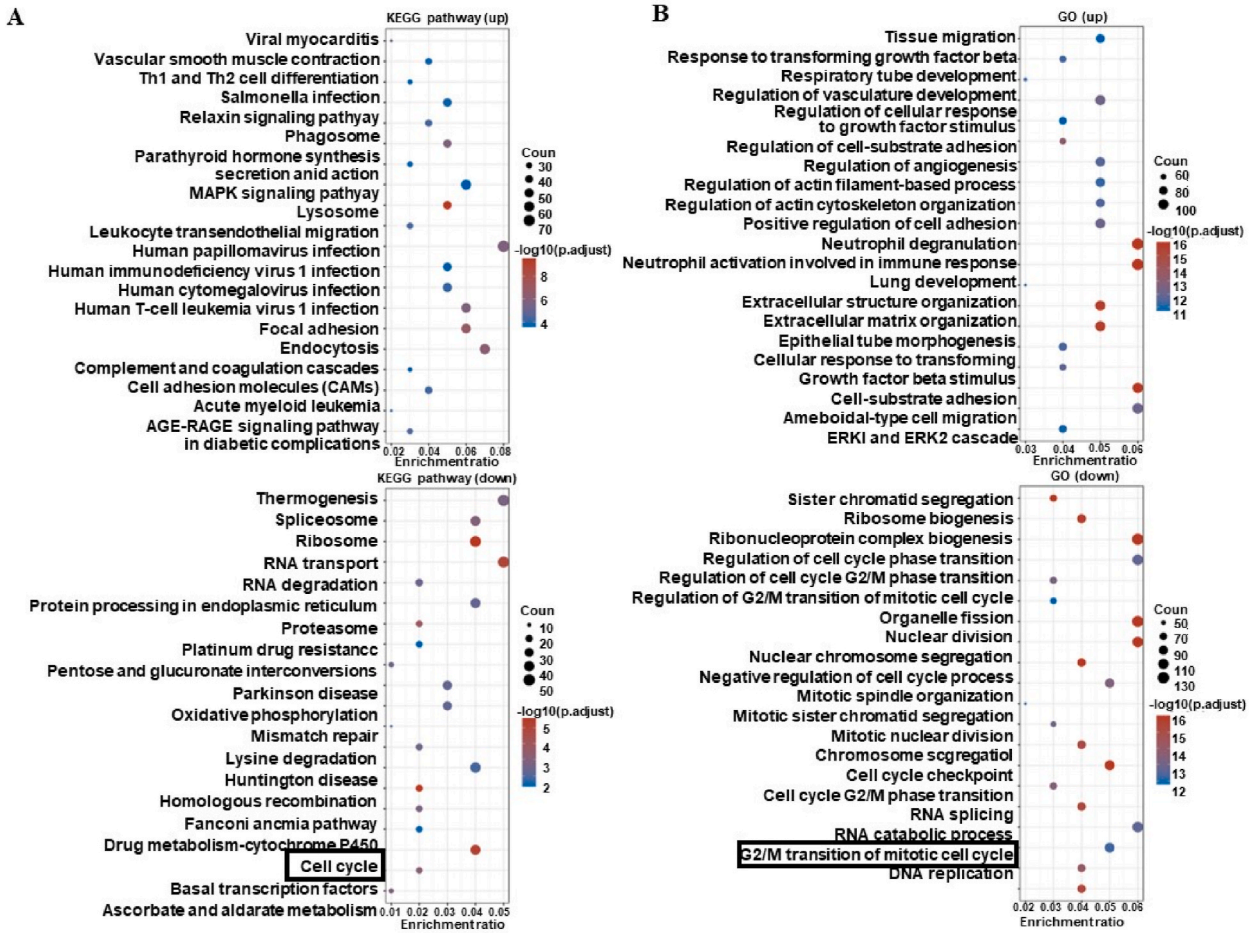

Fig. 2. GO and KEGG pathways enrichment analysis. (A) Visualization of the top 20 significant KEGG pathways enriched by the targeted genes in NSCLC, presented individually for up- and down-regulated patterns. The abscissa denotes the gene ratio, while the enriched pathways are displayed along the ordinate. (B) Illustration of the topmost GO enrichment pathways of targeted genes in NSCLC, presented in a similar manner. Colors denote the significance of differential enrichment, and the size of the circles reflects the number of genes; a larger circle indicates a greater number of genes.

utilized the bioinformatics tool ASSISTANT for Clinical Bioinformatics to analyze data within the GSE40275 dataset. We detected abnormal gene expression of CSRNP1 using various analytical methods, including box plots, PCA biplots, volcano maps, and heatmap analysis (Fig. 1A–D). he application of PCA to gene expression data from the GSE40275 dataset unveiled a clear separation between NSCLC patients and healthy controls (Fig. 1B). The differentially expressed genes (DEGs) identified through PCA exhibited a robust capability to discriminate between the two groups. This observation emphasizes the potential utility of these DEGs as reliable biomarkers for distinguishing NSCLC patients from healthy individual, offering valuable insights into the molecular signatures associated with the disease. Interestingly, the volcano biplots and heatmap revealed 1965 up-regulated genes and 2289 down-regulated genes (Fig. 1C and D). Among the 4254 up/down-regulated genes, CSRNP1 emerged as novel and was selected for further analysis based on its log fold change (FC) and significance values ($p < 0.0001$) between NSCLC (logFC: 1.7 ± 0.736) and healthy (logFC: 0.68 ± 0.514) groups. Notably, we observed a significant downregulation of CSRNP1 in NSCLC patients compared to healthy controls (Fig. 1E, $P < 0.0001$). This finding highlights the potential relevance of CSRNP1 as a molecular marker in distinguishing between individuals with NSCLC and healthy individuals.

3.2. *Pathways effected in NSCLC patients*

To elucidate the significant biological implications of key potential mRNAs and pathways, we conducted KEGG signaling pathway analysis. Additionally, gene ontology analysis was employed to unveil the potential targets of these mRNAs. The top 20 KEGG pathways, based on the upregulated or downregulated genes in NSCLC and healthy groups, were identified (Fig. 2A, $P < 0.05$). Similarly, the GO database was utilized to identify the top 20 significantly enriched pathways associated with the upregulated or downregulated genes (Fig. 2B, $P < 0.05$). Notably, the analysis from both pathways revealed a prominent enrichment of downregulated genes in cell cycle-related pathways, particularly associated with the G2/M phase transition. Overall, our analysis highlights a significant enrichment of downregulated genes in NSCLC, particularly impacting cell cycle-related pathways and the G2/M phase

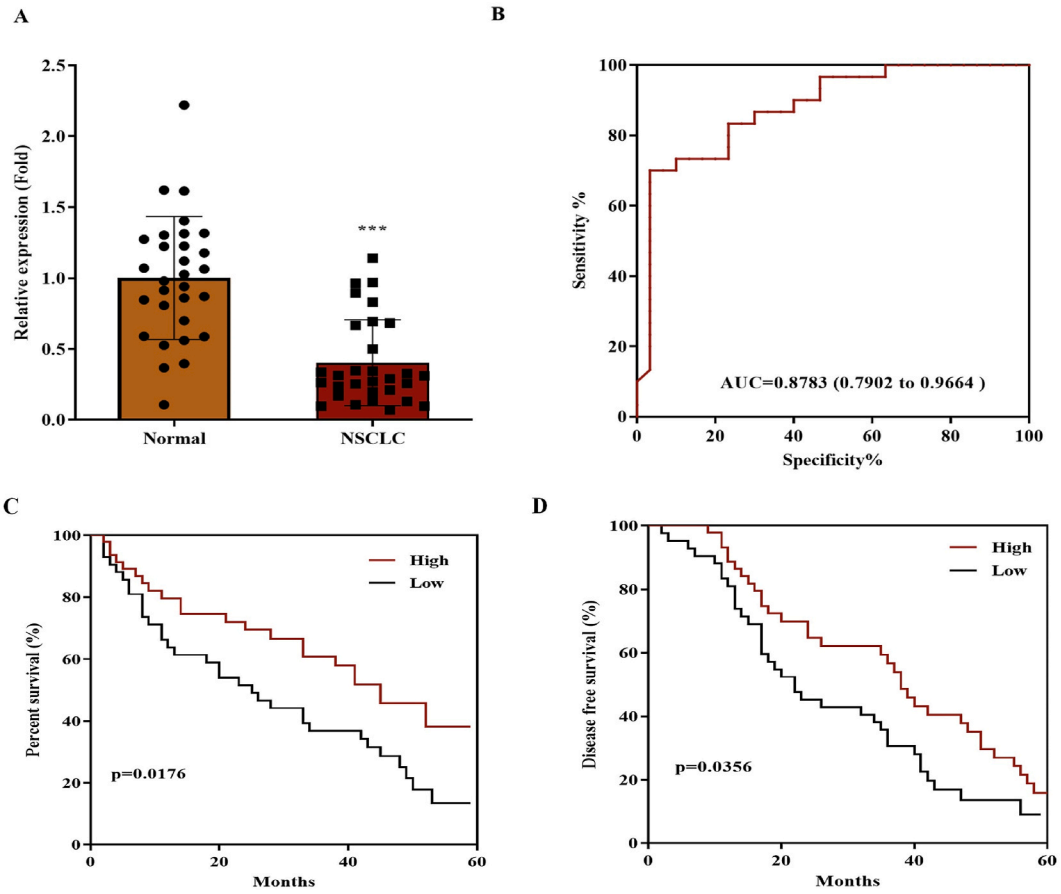

Fig. 3. The validation of CSRNP1 expression and Survival analyses of CSRNP1 in NSCLC patients. (A) The box plot represented the overall expression of CSRNP1 in NSCLC patients between adjacent tissue and tumor samples by RT-qPCR. Independent two-sample *t*-test was used. ***$p < 0.001$ vs normal. (B) The clinical significance of CSRNP1 in NSCLC patients was evaluated by ROC curve. (C–D) The survival graph represented association of high/low expression of CSRNP1 with overall survival (C) and disease-free survival (D) of NSCLC patients. Red: Higher expression, Black: Lower expression.

transition.

3.3. Validation of clinical significance of CSRNP1 in NSCLC patients

After investigating the pathways effected in NSCLC patients, we next aimed to validate expression of CSRNP1, explore its clinical implications, and assess prognostic value of CSRNP1. For this purpose, we conducted RT-qPCR analysis, ROC, and survival analysis. Our findings revealed a significant downregulation of CSRNP1 expression in NSCLC tumor tissues compared to healthy controls (Fig. 3A, $t = 6.2$, $P < 0.001$). The ROC curve for CSRNP1 demonstrated an impressive Area Under the Curve (AUC) of 0.8783 (95% CI = 0.7902–0.9664, $P < 0.05$), underscoring its potential as a biomarker for distinguishing NSCLC patients and healthy controls (Fig. 3B). This highlights both the validated downregulation of CSRNP1 in NSCLC and its promising clinical significance as a diagnostic indicator. We conducted additional survival analysis to further illuminate CSRNP1's role in NSCLC. Higher CSRNP1 expression, in comparison to lower levels, significantly correlated with increased overall survival in NSCLC patients, supported by Kaplan-Meier survival analysis (Fig. 3C, $P < 0.05 = 0.0176$). Moreover, heightened CSRNP1 expression was associated with a greater proportion of disease-free survival (Fig. 3D, $P < 0.050356$). Collectively, our survival analyses underscore potential of CSRNP1 as a favorable prognostic marker for NSCLC patients.

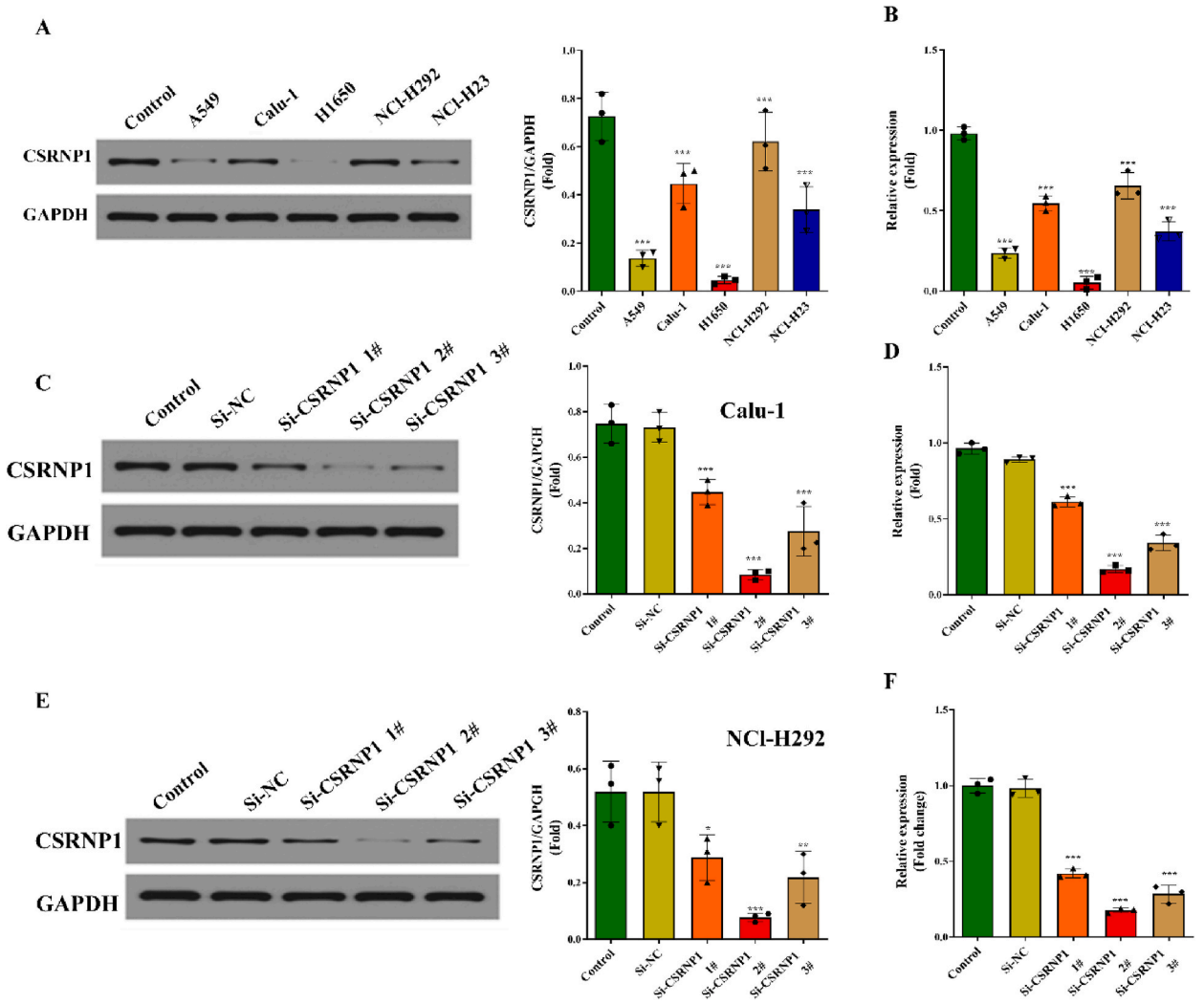

Fig. 4. Expression of CSRNP1 in control and cancer cell lines. *(A, B) Western blots and qPCR showed differential protein expressions and mRNA of CSRNP1 in five NSCLC cell lines and control cell line (BEAS-2B). Western blots have been provided as supplementary material.One-way ANOVA was conducted. ***P < 0.001 vs control. (C–F) Calu-1 and NCI–H292 demonstrated lower expression of CSRNP1 after silencing with Si-CSRNP1 (1#,2#,3#) compared with Si-NC and control groups. All experiments were repeated in triplicate with sample size n = 3 for robustness and reliability. One-way ANOVA was conducted. *P < 0.05, **P < 0.01, ***P < 0.001 vs si-NC.*

3.4. Expression of CSRNP1 in NSCLC cell lines

Following the validation of clinical significance of CSRNP1 in NSCLC patients, we explored protein and CSRNP1 mRNA expression in NSCLC cancerous cell lines (Calu-1, A549, H1650, NCI–H292, NCI–H23) and the BEAS-2 control cell line. Our results demonstrated a significant down-regulation of CSRNP1 in NSCLC cells at both protein (Fig. 4A, $F = 30.3$, $P < 0.001$) and mRNA levels (Fig. 4B, $F = 116.8$, $P < 0.001$). Calu-1 and NCI–H292 exhibited higher CSRNP1 expression, leading us to conduct subsequent experiments on these two cell lines. Silencing CSRNP1 in these cells significantly reduced CSRNP1 protein (Calu-1: $F = 46.1$, $P < 0.001$; NCI–H292: $F = 15.8$, $P < 0.001$) and mRNA expression (Calu-1: $F = 256.2$, $P < 0.001$; NCI–H292: $F = 193.7$, $P < 0.001$) in the Si-CSRNP1 groups (#1, #2, #3) as depicted in Fig. 4C–F ($P < 0.05$). Notably, siRNA-2 exhibited superior efficacy among the tested siRNAs, prompting its selection for subsequent experiments. Overall, our in vitro investigations revealed a significant downregulation of CSRNP1 in various NSCLC cell lines as compared to control cell line.

3.5. Effects of CSRNP1 silencing on migration, invasion, and cell viability in Calu-1 and NCI–H292 cells

The impact of CSRNP1 silencing on Calu-1 and NCI–H292 cell migration, invasion, and viability were subsequently assessed. The wound-healing assay provides a cost-effective and straightforward method for investigating directional cell migration in vitro [31]. The transwell invasion assay, widely employed to assess the invasive response of endothelial cells and stands out for its high detection sensitivity [32]. The wound-healing assay demonstrated increased wound closure in Calu-1 ($F = 18.5$, $P < 0.001$) and NCI–H292 ($F = 16.5$, $P < 0.001$) cells in the Si-CSRNP1 group compared to Si-NC, indicating enhanced migration capacity in Si-CSRNP1 cells (Fig. 5A and B, $P < 0.05$). Transwell assay revealed elevated invasion in Si-CSRNP1 Calu-1 ($F = 12.4$, $P < 0.001$) and NCI–H292 cells ($F = 13.9$, $P < 0.001$) compared to Si-NC, signifying accelerated invasion (Fig. 5C,D, $P < 0.05$). MTT assay showed increased viability in CSRNP1-silenced Calu-1 ($F = 10.2$, $P < 0.001$) and NCI–H292 ($F = 11.1$, $P < 0.001$) cells, suggesting a vital role in NSCLC malignancy (Fig. 5E, $P < 0.05$). Overall, these findings indicate that downregulation of CSRNP1 in NSCLC cells not only promotes migration and invasion but also significantly increases cell viability, suggesting a crucial role in the malignancy of these cells.

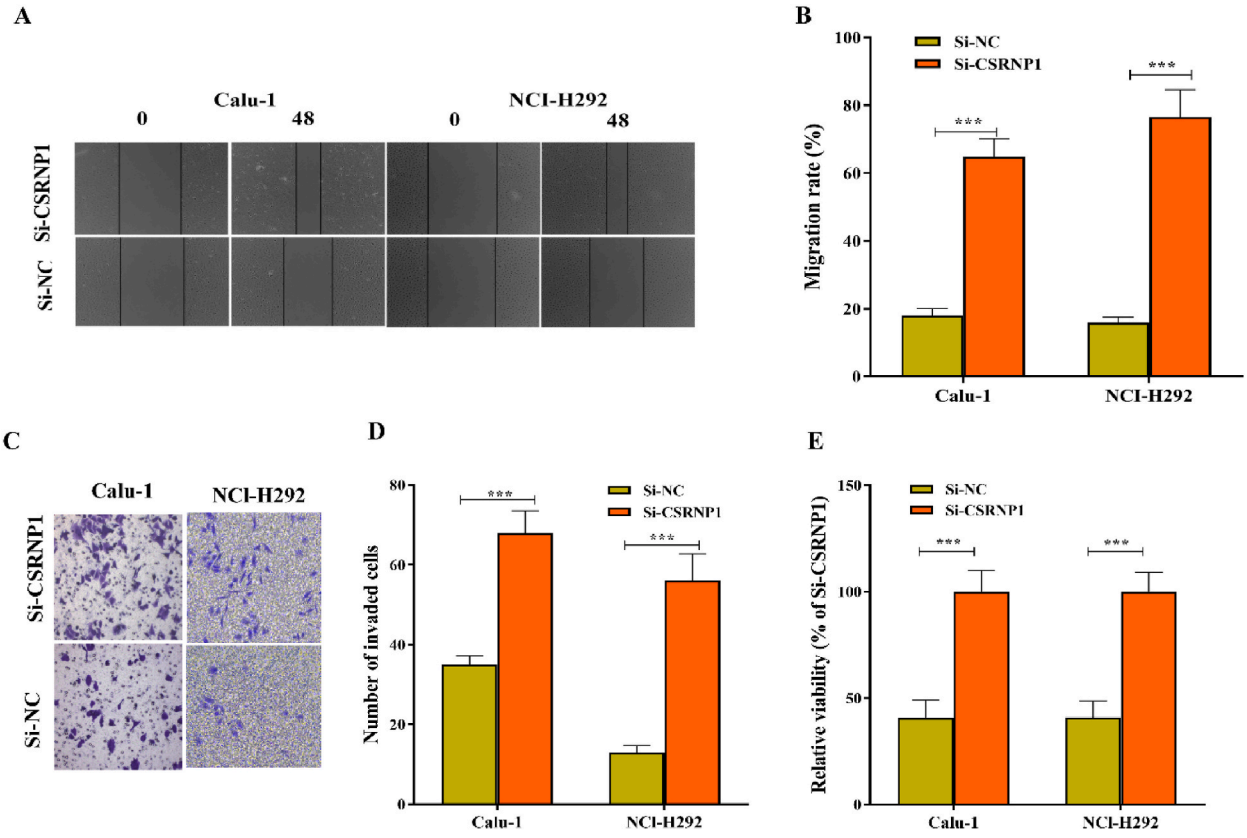

Fig. 5. The biological mechanism of CSRNP1 silencing in Calu-1 and NCI–H292 cells. (A, B) A wound-healing assay was performed to evaluate the migratory ability of Calu-1 and NCI–H292 cells after transfection with Si-CSRNP1 and Si-NC. (C, D) Transwell assay was performed on Calu-1 and NCI–H292 cells and showed invasion capability of cells. (E) MTT assay showed the cell viability of siRNAs transfected cells. Independent two-sample *t*-test was used. ***$P < 0.001$ vs Si-NC.

3.6. CSRNP1 silencing promoted the activation of the Akt/MDM2/p53 pathway

Given that downregulated genes in NSCLC were predominantly enriched in cell cycle-related pathways and associated with the G2/M phase transition (Fig. 2), we explored the impact of silencing CSRNP1 in Calu-1 cells on downregulated signaling pathways of cell cycle. The Akt/MDM2/p53 pathway is reported to be involved in the regulation of cell growth and cell cycle in lung cancer [33]. Key proteins in the G2/M phase transition, including CDK1 and Cyclin B, were specifically targeted for investigation [34]. Consequently, protein levels of *p*-AKT, AKT, *p*-MDM2, MDM2, p53, CDK1, and Cyclin B were assessed. The results from Western blot analysis revealed a significant increase in the protein expression of *p*-Akt/Akt, *p*-MDM2/MDM2, CDK1, and Cyclin B, accompanied by a decrease in p53 expression in CSRNP1-silenced Calu-1 cells compared to the blank or Si-NC group (Fig. 6A and B). These findings suggest that the knockdown of CSRNP1 promotes the activation of the Akt/MDM2/p53 signaling pathway in vitro.

4. Discussion

For a decade, extensive studies have demonstrated that lung cancer incidence is notably soaring, with huge influence on the health and life of patients [35,36]. It is the second most frequently diagnosed cancer in both males and females and has the highest mortality in both sexes [37]. Compared to other common cancers, patients with lung cancer have the highest risk of malignancy-associated suicide [38].

NSCLC is the most common kind of lung cancer characterized by high-rise incidence and accounts for 85% of all lung cancer cases [39,40]. For patients at early-stage disease, NSCLC poses a significant clinical challenge with a propensity for distant metastasis and early relapse [41]. NSCLC has a 5-year survival rate of 65% in localized disease and 9% in metastatic disease [42]. Due to high disease burden, it is critical to find new molecules and associated pathways involved in pathogenesis of NSCLC to curb the disease.

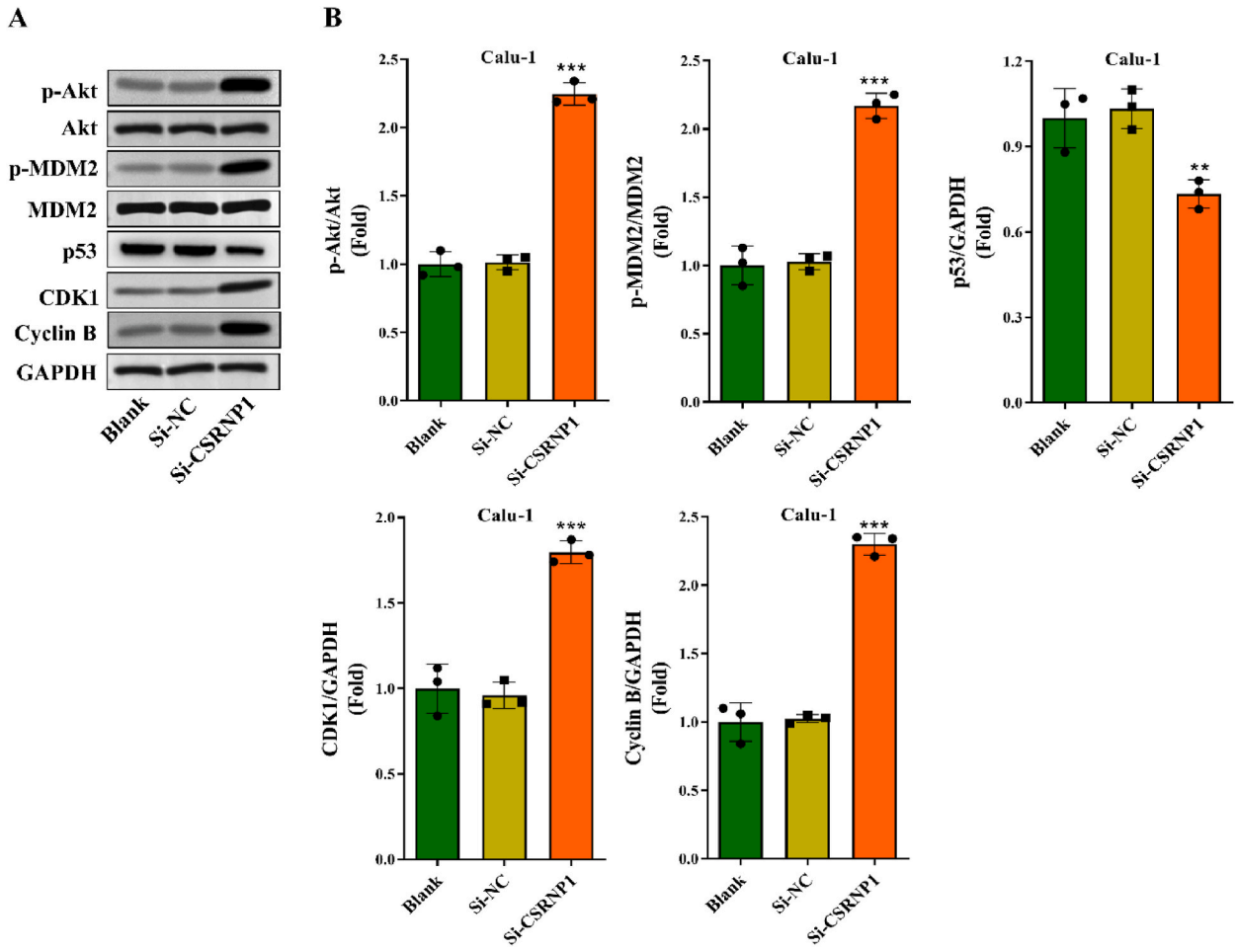

Fig. 6. Activation of the Akt/MDM2/p53 pathway in CSRNP1-silenced Calu-1 cells. (A, B) Western blot was performed to detect the protein expression of *p*-Akt, Akt, *p*-MDM2, MDM2, p53, CDK1, and Cyclin B in Calu-1 cells (Uncropped blots have been provided as supplementary material). One-way ANOVA was conducted. *P < 0.05, **P < 0.01. All experiments were repeated in triplicate with sample size n = 3 for robustness and reliability.

Interestingly, in the last two decades, significant gains have been made in understanding the genetic mutations and underlying biomolecular mechanisms, however still the disease burden is still high.

CSRNP1 has been suggested as an immediate early gene [43] that is linked to a special AGAGTG sequence and comprised of cysteine and serine-rich based domains. In articular chondrocytes, CSRNP1 serves as transcriptional regulator and upregulates the expression of matrix metalloproteinases [44]. Importantly, expression of CSRNP1 could be highly induced by IL-2 in mouse T lymphocytes in an *in vivo* model [16]. TCGA cohort indicated that CSRNP1 is downregulated in tumor tissues, but interestingly its down-regulation predicts a better prognosis in NSCLC patients [25]. Study by Wang Z et al. has proposed a seven gene prognostic signature in lung squamous cell carcinoma, and CSRNP1 was included as a prognostic biomarker [26]. CSRNP1 gene is a prognostic biomarker for clear cell renal cell carcinoma, and high expression of CSRNP1 is correlated with enhanced immune response and improved survival outcomes [15]. Meanwhile, CSRNP1 upregulation is reported to disrupt cell-cycle progression by downregulating the activity of Cdk1 and promotes apoptosis through a JNK-dependent manner [45]. These findings might facilitate our understanding of the role of CSRNP1 in the progression of various types of diseases. However, its precise role in patients with NSCLC requires investigation. In this study, we used systematic bioinformatics tool to show that CSRNP1 could be an important prognostic factor for NSCLC, and its downregulation in tumor tissue of NSCLC patients was confirmed. Moreover, our study revealed that CSRNP1 protein was valuable in differentiating tumor and adjacent tissues of NSCLC significantly with an AUC of 0.8783, and higher expression of CSRNP1 was related with longer OS and DFS in NSCLC patients, consistent with a previous study [25]. Therefore, CSRNP1 protein could serve as a novel biomarker for diagnosing NSCLC patients and predicting their survival.

The present work explored the biological functions of CSRNP1 in NSCLC cells by transfecting Si-CSRNP1 plasmids. Herein, knockdown of CSRNP1 promoted NSCLC cell proliferation, migration, as well as invasion. Thus, CSRNP1 may not only act in the biological mechanism of NSCLC but also work as a promising therapeutic target of NSCLC.

Interestingly, GO and KEGG pathways indicated several deregulated pathways especially cell cycle and DNA replication pathways. We proceeded to investigate the protein levels of *p*-Akt, p53, *p*-MDM2, CDK1, and Cyclin B, which are involved in various cellular processes, including cell cycle regulation [46–48]. The Akt pathway is implicated in the progression of NSCLC [49,50]. P53 is a tumor suppressor and plays critical role in cell cycle arrest [51–54]. Akt inhibits p53 expression by phosphorylating and facilitating the nuclear translocation of oncogene MDM2 [55–57]. MDM2 inhibits p53-mediated senescence and promotes tumorigenesis [58]. Interestingly, Akt enhances MDM2-mediated ubiquitination and degradation of p53 [59–61]. Previous studies have also revealed that this signaling pathway participates in the regulation of cancer cell growth, cell cycle arrest, and cell apoptosis in lung cancer [33,62]. In our study, we observed a significant increase in the levels of *p*-Akt/Akt, *p*-MDM2, CDK1, and Cyclin B in CSRNP1-silenced NSCLC cells, accompanied by a concomitant decrease in p53 levels. These findings suggest that the tumor suppressor CSRNP1 may play a dual role by modulating the Akt/MDM2/p53 pathway in NSCLC. A previous study also suggests that CSRNP1 is associated with the Akt pathway [63], yet the precise mechanism remains elusive. Considering role of CSRNP1 as a transcriptional factor for MMP1 [44], and the known role of MMP-1 in targeting the Akt pathway to enhance cell survival and inhibit apoptosis [64], it is plausible that CSRNP1 might regulate the Akt pathway, thereby impacting NSCLC progression through MMP1 modulation. This presents a potential avenue for future research.

Nevertheless, this study has some limitations. The initial screening of CSRNP1 using the GEO online database may introduce potential bias in microarray results or sample selection. Additionally, further investigations involving a larger cohort are warranted to validate our findings. Furthermore, our assessment of CSRNP1 was confined to tissue samples and NSCLC cell lines but lacked validation of long-term effects of CSRNP1 expression in patients. Future studies incorporating animal models will be performed to elucidate the *in vivo* role of CSRNP1 in NSCLC progression. These steps are crucial for a comprehensive understanding of CSRNP1's potential as a therapeutic target and its relevance in the clinical management of NSCLC.

In conclusion, our study utilized bioinformatics tools to unveil the downregulation of CSRNP1 in NSCLC, which was subsequently validated in a cohort of 30 patients. We elucidated the clinical significance of CSRNP1 in NSCLC, and established its relevance to diagnosis and survival in NSCLC. Given the close association of NSCLC tumorigenesis and metastasis with cell proliferation, migration, and invasion [65], our findings further underscored that siRNA-induced CSRNP1 silencing exerts a promoting effect on NSCLC cell proliferation, migration, and invasion. This suggests that CSRNP1 not only serves as a diagnostic and prognostic biomarker for NSCLC but also holds therapeutic potential as a clinical target for this cancer.

Ethical approval

The current research ethics was reviewed and approved by the Ethical Committee of Nanjing medical university under approval number #NMU-2023AAUG.

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NA.

Data availability statement

The additional data may be available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Zhongneng Xu: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Hao Zhou:** Writing – original draft, Project administration, Methodology, Data curation, Conceptualization. **Yonggang Luo:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Nunu Li:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Sheng Chen:** Supervision, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Nothing to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28412.

References

[1] W.D. Travis, et al., The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification, J. Thorac. Oncol. 10 (9) (2015) 1243–1260.
[2] N. Wang, et al., [Research progress of antibody-drug conjugates in advanced non-small cell lung cancer], Zhongguo Fei Ai Za Zhi 25 (3) (2022) 214–218.
[3] J. Yong, et al., High expression of Stabilin-2 predicts poor prognosis in non-small-cell lung cancer, Bioengineered 12 (1) (2021) 3426–3433.
[4] H. Sung, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: a cancer journal for clinicians 71 (3) (2021) 209–249.
[5] C. Xia, et al., Cancer statistics in China and United States, 2022: profiles, trends, and determinants, Chin. Med. J. 135 (5) (2022) 584–590.
[6] F. Kocher, et al., Longitudinal analysis of 2293 NSCLC patients: a comprehensive study from the TYROL registry, Lung Cancer 87 (2) (2015) 193–200.
[7] K. Król, et al., Non-small cell lung cancer treatment with molecularly targeted therapy and concurrent radiotherapy-A review, Int. J. Mol. Sci. 24 (6) (2023).
[8] A. Sahgal, et al., Stereotactic body radiotherapy versus conventional external beam radiotherapy in patients with painful spinal metastases: an open-label, multicentre, randomised, controlled, phase 2/3 trial, Lancet Oncol. 22 (7) (2021) 1023–1033.
[9] A. Casal-Mouriño, et al., Epidemiology of stage III lung cancer: frequency, diagnostic characteristics, and survival, Transl. Lung Cancer Res. 10 (1) (2021) 506–518.
[10] Z. Liang, et al., MiR-187 suppresses non-small-cell lung cancer cell proliferation by targeting FGF9, Bioengineered 11 (1) (2020) 70–80.
[11] L. Wei, et al., Aberrant expression of HIF3A in plasma of patients with non-small cell lung cancer and its clinical significance, J. Clin. Lab. Anal. 35 (8) (2021) e23889.
[12] Y. Xiao, et al., Recent progress in targeted therapy for non-small cell lung cancer, Front. Pharmacol. 14 (2023) 1125547.
[13] J. Wu, Z. Lin, Non-small cell lung cancer targeted therapy: drugs and mechanisms of drug resistance, Int. J. Mol. Sci. 23 (23) (2022).
[14] A.A. Thai, et al., Lung cancer, Lancet 398 (10299) (2021) 535–554.
[15] H. Zhang, X. Qiu, G. Yang, The CSRNP gene family serves as a prognostic biomarker in clear cell renal cell carcinoma, Front. Oncol. 11 (2021) 620126.
[16] S. Gingras, et al., Characterization of a family of novel cysteine- serine-rich nuclear proteins (CSRNP), PLoS One 2 (8) (2007) e808.
[17] J. Espina, et al., csrnp1a is necessary for the development of primitive hematopoiesis progenitors in zebrafish, PLoS One 8 (1) (2013) e53858.
[18] J. Chen, et al., Identifying candidate genes for Type 2 Diabetes Mellitus and obesity through gene expression profiling in multiple tissues or cells, J. Diabetes Res. 2013 (2013) 970435.
[19] T.H. Chiang, et al., Mass eradication of Helicobacter pylori to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands, Gut 70 (2) (2021) 243–250.
[20] H. Ishiguro, et al., Identification of AXUD1, a novel human gene induced by AXIN1 and its reduced expression in human carcinomas of the lung, liver, colon and kidney, Oncogene 20 (36) (2001) 5062–5066.
[21] W.H. Xu, et al., Prognostic implication and functional annotations of Rad50 expression in patients with prostate cancer, J. Cell. Biochem. 121 (5–6) (2020) 3124–3134.
[22] A. Kobelyatskaya, et al., Differentially methylated CpG sites associated with the high-risk group of prostate cancer, Journal of Integrative Bioinformatics 17 (4) (2020).
[23] M.Q. Yang, et al., A gene module-based eQTL analysis prioritizing disease genes and pathways in kidney cancer, Comput. Struct. Biotechnol. J. 15 (2017) 463–470.
[24] B. Xu, et al., Prognostic genes of hepatocellular carcinoma based on gene coexpression network analysis, J. Cell. Biochem. 120 (7) (2019) 11616–11623.
[25] J. Pang, et al., Integrating Single-cell RNA-seq to construct a Neutrophil prognostic model for predicting immune responses in non-small cell lung cancer, J. Transl. Med. 20 (1) (2022) 531.
[26] Z. Wang, et al., Identification of seven-gene signature for prediction of lung squamous cell carcinoma, OncoTargets Ther. 12 (2019) 5979–5988.
[27] C.G. Feijóo, et al., Cystein-serine-rich nuclear protein 1, Axud1/Csrnp1, is essential for cephalic neural progenitor proliferation and survival in zebrafish, Dev. Dynam. 238 (8) (2009) 2034–2043.
[28] K. Korb, et al., Inhibition of AXUD1 attenuates compression-dependent apoptosis of cementoblasts, Clin. Oral Invest. 20 (9) (2016) 2333–2341.
[29] X. Ye, et al., Axin1 up-regulated 1 accelerates stress-induced cardiomyocytes apoptosis through activating Wnt/β-catenin signaling, Exp. Cell Res. 359 (2) (2017) 441–448.
[30] S. Liu, et al., Human embryonic germ cells isolation from early stages of post-implantation embryos, Cell Tissue Res. 318 (3) (2004) 525–531.
[31] L.G. Rodriguez, X. Wu, J.L. Guan, Wound-healing assay, Methods Mol. Biol. 294 (2005) 23–29.
[32] C.R. Justus, et al., Transwell in vitro cell migration and invasion assays, Methods Mol. Biol. 2644 (2023) 349–359.
[33] Y. Zhao, et al., Germacrone induces lung cancer cell apoptosis and cell cycle arrest via the Akt/MDM2/p53 signaling pathway, Mol. Med. Rep. 23 (6) (2021).
[34] Z. Wang, et al., Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression, Dev. Cell 29 (2) (2014) 217–232.

[35] M. Charakidis, M. Boyer, Targeting MET and EGFR in NSCLC-what can we learn from the recently reported phase III trial of onartuzumab in combination with erlotinib in advanced non-small cell lung cancer? Transl. Lung Cancer Res. 3 (6) (2014) 395–396.
[36] H.L. Jiang, L.M. Jiang, W.D. Han, Wnt/beta-catenin signaling pathway in lung cancer stem cells is a potential target for the development of novel anticancer drugs, J BUON 20 (4) (2015) 1094–1100.
[37] R.L. Siegel, et al., Cancer statistics, 2023, CA Cancer J Clin 73 (1) (2023) 17–48.
[38] M. Rahouma, et al., Lung cancer patients have the highest malignancy-associated suicide rate in USA: a population-based analysis, Ecancermedicalscience 12 (2018) 859.
[39] F. Yang, The expression and mechanism of Sirt1 and AMPK in nonsmall cell lung cancer, J BUON 23 (1) (2018) 106–110.
[40] N. Duma, R. Santana-Davila, J.R. Molina, Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment, Mayo Clin. Proc. 94 (8) (2019) 1623–1640.
[41] G.A. Woodard, et al., Lung Cancer Staging and Prognosis, 2016, pp. 47–75.
[42] J. McLaughlin, J. Berkman, P. Nana-Sinkam, Targeted therapies in non-small cell lung cancer: present and future, Fac Rev 12 (2023) 22.
[43] J.J. Hutton, et al., Microarray and comparative genomics-based identification of genes and gene regulatory regions of the mouse immune system, BMC Genom. 5 (2004) 82.
[44] C.D. Macdonald, et al., Cytokine-induced cysteine- serine-rich nuclear protein-1 (CSRNP1) selectively contributes to MMP1 expression in human chondrocytes, PLoS One 13 (11) (2018) e0207240.
[45] A. Glavic, et al., Drosophila Axud1 is involved in the control of proliferation and displays pro-apoptotic activity, Mech. Dev. 126 (3–4) (2009) 184–197.
[46] A. Glaviano, et al., PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer, Mol. Cancer 22 (1) (2023) 138.
[47] L. Gu, et al., The MDM2 RING finger is required for cell cycle-dependent regulation of its protein expression, FEBS Lett. 544 (1–3) (2003) 218–222.
[48] G. Cullot, et al., Cell cycle arrest and p53 prevent ON-target megabase-scale rearrangements induced by CRISPR-Cas9, Nat. Commun. 14 (1) (2023) 4072.
[49] S. Heavey, K.J. O'Byrne, K. Gately, Strategies for co-targeting the PI3K/AKT/mTOR pathway in NSCLC, Cancer Treat Rev. 40 (3) (2014) 445–456.
[50] B. Chen, et al., Fangchinoline inhibits non-small cell lung cancer metastasis by reversing epithelial-mesenchymal transition and suppressing the cytosolic ROS-related Akt-mTOR signaling pathway, Cancer Lett. 543 (2022) 215783.
[51] T.T.T. Phan, et al., Tumor suppressor p53 mediates interleukin-6 expression to enable cancer cell evasion of genotoxic stress, Cell Death Discovery 9 (1) (2023) 340.
[52] G. Divita, et al., Abstract 550: p53 rescue of tumor suppressor function with ADGN-531 causes tumor regression both as single agent and in combination with PARPi and KRASG12Cinhibitors, Cancer Res. 83 (7_Supplement) (2023), 550-550.
[53] K. Engeland, Cell cycle regulation: p53-p21-RB signaling, Cell Death Differ. 29 (5) (2022) 946–960.
[54] R. Mehdizadeh, et al., P53 status, and G2/M cell cycle arrest, are determining factors in cell-death induction mediated by ELF-EMF in glioblastoma, Sci. Rep. 13 (1) (2023) 10845.
[55] A.G. Abraham, E. O'Neill, PI3K/Akt-mediated regulation of p53 in cancer, Biochem. Soc. Trans. 42 (4) (2014) 798–803.
[56] L.D. Mayo, D.B. Donner, A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus, Proc. Natl. Acad. Sci. U. S. A. 98 (20) (2001) 11598–11603.
[57] S. Singh, et al., Human oncoprotein MDM2 activates the Akt signaling pathway through an interaction with the repressor element-1 silencing transcription factor conferring a survival advantage to cancer cells, Cell Death Differ. 20 (4) (2013) 558–566.
[58] L. Chibaya, et al., Mdm2 phosphorylation by Akt regulates the p53 response to oxidative stress to promote cell proliferation and tumorigenesis, Proc. Natl. Acad. Sci. U. S. A. 118 (4) (2021).
[59] Y. Ogawara, et al., Akt enhances Mdm2-mediated ubiquitination and degradation of p53*, J. Biol. Chem. 277 (24) (2002) 21843–21850.
[60] J.C. Marine, G. Lozano, Mdm2-mediated ubiquitylation: p53 and beyond, Cell Death Differ. 17 (1) (2010) 93–102.
[61] P.-J. Tsai, et al., Akt: a key transducer in cancer, J. Biomed. Sci. 29 (1) (2022) 76.
[62] Z. Liang, et al., HAX1 enhances the survival and metastasis of non-small cell lung cancer through the AKT/mTOR and MDM2/p53 signaling pathway, Thorac Cancer 11 (11) (2020) 3155–3167.
[63] K. Zhao, et al., RNA sequencing-based identification of the regulatory mechanism of microRNAs, transcription factors, and corresponding target genes involved in vascular dementia, Front. Neurosci. 16 (2022) 917489.
[64] B.X.J. Tune, et al., Matrix metalloproteinases in chemoresistance: regulatory roles, molecular interactions, and potential inhibitors, Journal of Oncology 2022 (2022) 3249766.
[65] Q.R. Guo, et al., Jaceosidin inhibits the progression and metastasis of NSCLC by regulating miR-34c-3p/Integrin α2β1 axis, Heliyon 9 (5) (2023) e16158.