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CHPF promotes lung adenocarcinoma proliferation and anti-apoptosis *via* the MAPK pathway

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ABSTRACT

Objective: Recent studies have shown that Chondroitin polymerizing factor (CHPF) is abnormally expressed in malignant tumors, however, the expression of CHPF in lung adenocarcinoma (LUAD) has not been reported. In this study, the relationship of CHPF and LUAD will be explored.

Methods: Differential genes present in LUAD were screened by bioinformatics analysis. The expression status of CHPF in LUAD tissues and cell lines were deteced by Western blotting or Real-time Quantitative Polymerase Chain Reaction Detecting System (qPCR), and the relationship between CHPF and prognosis of LUAD patients was analyzed. CHPF was effectively silenced in LUAD cell lines by lentivirus- mediated methods. The effect of CHPF on proliferation, cell cycle progression and apoptosis of cancer cells was assessed. We further determined the role of CHPF in tumor growth *in vivo* by using xenograft LUAD tumor models. Western blotting assay was performed to assess the expression changes of MAPK signaling pathway.

Results: We found that CHPF is highly expressed in LUAD tissues and cell lines. *In vitro* experiments, CHPF knockdown in LUAD cells can effectively inhibit proliferation and promote apoptosis of cancer cell. *In vivo* experiment, tumor growth was markedly inhibited by CHPF knockdown in the xenograft model of LUAD. Notably, CHPF also could promote tumor progression by regulating MAPK pathway.

Conclusion: CHPF can promote the proliferation and antiapoptosis of LUAD cells, which is promising to become a potential target for LUAD treatment.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths globally. According to epidemiological surveys, there are an estimated 1.6 million new cases and 1.4 million deaths each year [1]. In China, lung cancer ranks the first among all malignancies in terms of incidence and mortality [2]. The most common pathological type of lung cancer is lung adenocarcinoma (LUAD). Most patients with LUAD are diagnosed at advanced stages. Despite advances in molecularly targeted drugs, which have improved the survival time of patients with sensitive mutations in driving genes, the five-year survival rate for patients with advanced LUAD is still less than 15%. Therefore, it is crucial to find new therapeutic targets.

Chondroitin polymerizing factor (CHPF), composed of 775 amino acids, is a type II transmembrane protein, with 23% amino acid homology with human chondroitin synthetase (ChSy). The CHPF gene is located on the human chromosome 2q35-q36 loci, spanning four exon regions. Recent studies have shown that CHPF is abnormally expressed in malignant tumors, including glioma and colorectal cancer [3,4]. However, the expression of CHPF in LUAD is still unclear. In this study, we assessed the biological role of CHPF in the pathogenesis of LUAD by integrating The Cancer Genome Atlas (TCGA) database and experimental results.

2. Materials and methods

2.1. Bioinformatic analysis

There are several types of tumor data in TCGA. For the expression of the target gene, RNAseq data were selected for analysis. Data were cataloged in Microsoft Excel and screened for major locations of tumors to exclude non-LUAD patients. A total of 521 LUAD samples with

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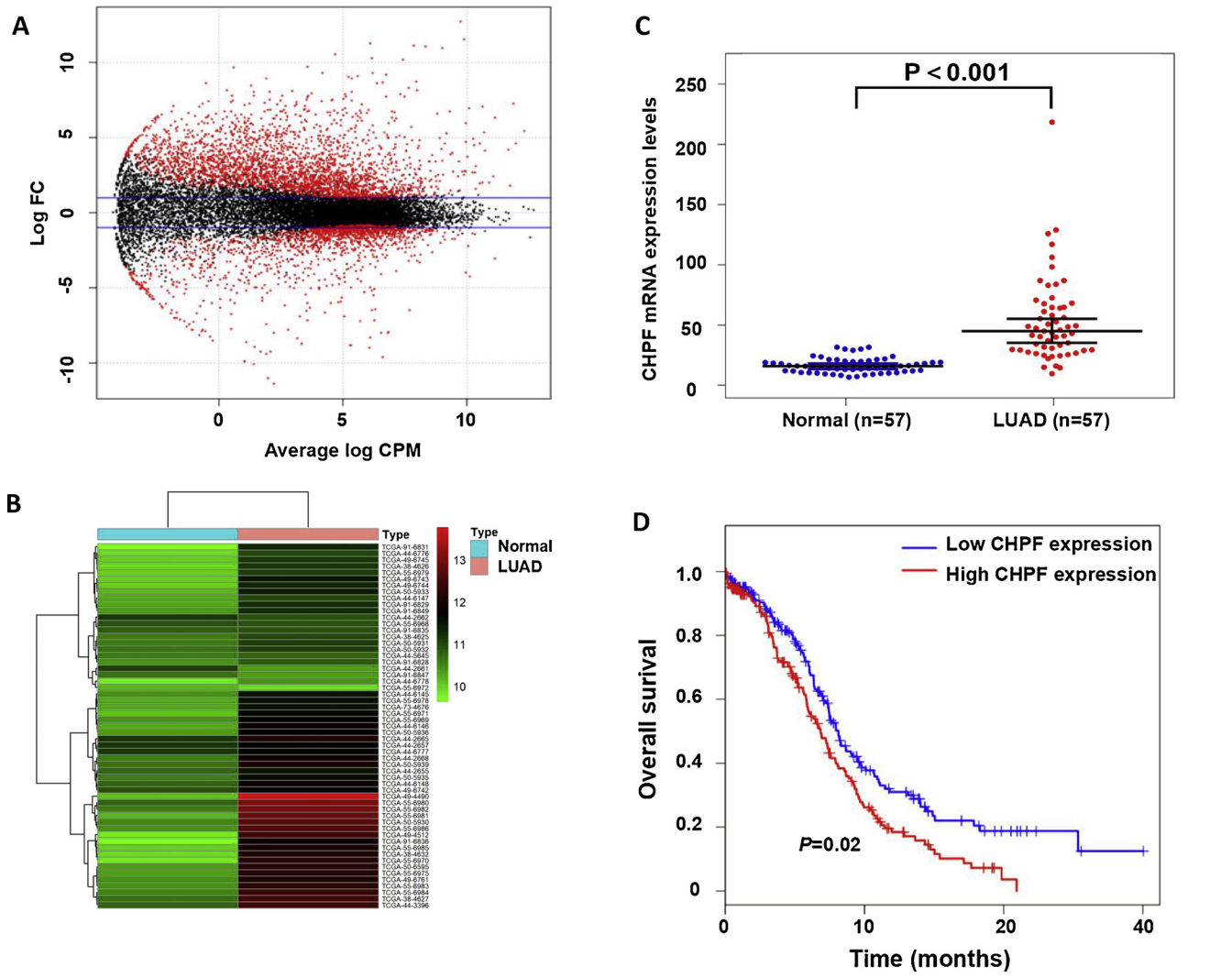

Fig. 1. (A) Volcano plot showed gene expression profiles in LUAD samples, the red dots indicate the genes which presence differential expression. (B) The heat map specifically shows the expression level of CHPF in 57 pairs of samples, red represent high expression of CHPF in this sample, and green represent low expression of CHPF in this sample. (C) Scatter-plot of CHPF mRNA expression in LUAD tissues and normal lung tissues. (D) The Kaplan-Meier curve predicts a worse prognosis for LUAD patients with high CHPF expression. Note: CHPF means chondroitin polymerizing factor; LUAD means lung adenocarcinoma.

available data were found in the TCGA database, 504 of which had accessible pathological information. Among them were 57 pairs of samples with matched (cancerous and paracancerous) RNAseq data and pathological information. Log_2 was used to calculate the fold change in differential gene expression and to generate volcano maps. Differential genes were analyzed by bidirectional hierarchical clustering using the pheatmap R package (https://cran.r-project.org/web/packages/pheatmap/). In the clustering map, red represented genes with high expression in the sample, and green represented genes with low expression. Student's *t* test was used to determine the difference in target gene expression between LUAD and normal tissues. Kaplan-Meier plotter database was used to analyze the correlation between target gene expression and prognosis in patients with LUAD. Data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used to perform Gene Set Enrichment Analysis (GSEA) for signal pathways. To investigate target gene-related signaling pathways in LUAD progression, we uploaded the total standardized expression data to GSEA v2.2.

2.2. Clinical samples and cell lines

A total of 34 cases of paraffin-embedded LUAD samples and corresponding adjacent tissues were collected from the First Affiliated Hospital of Lanzhou University between January 2010 and December 2013. All patients were pathologically diagnosed with complete clinical data. The average age was 44.7 ± 15.1 years (35–68 years). Of the 34 patients with LUAD, 19 were male and 15 were female. Patients' tumors were staged according to the tumor node metastasis (TNM) staging system jointly established by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC). None of the patients had ever received chemotherapy, radiotherapy, or biotherapy before sample collection. Informed consent form was obtained from all patients or their family members. The study was approved by the Ethics Committee of The First Hospital of Lanzhou University.

The human LUAD cell lines SPCA1, A549, 95-D, and H1299 and the normal lung epithelial cells BEAS-2B were purchased from the Shanghai Branch of the Chinese Academy of Sciences. All cells were maintained in 1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 incubator. The medium was changed every three days.

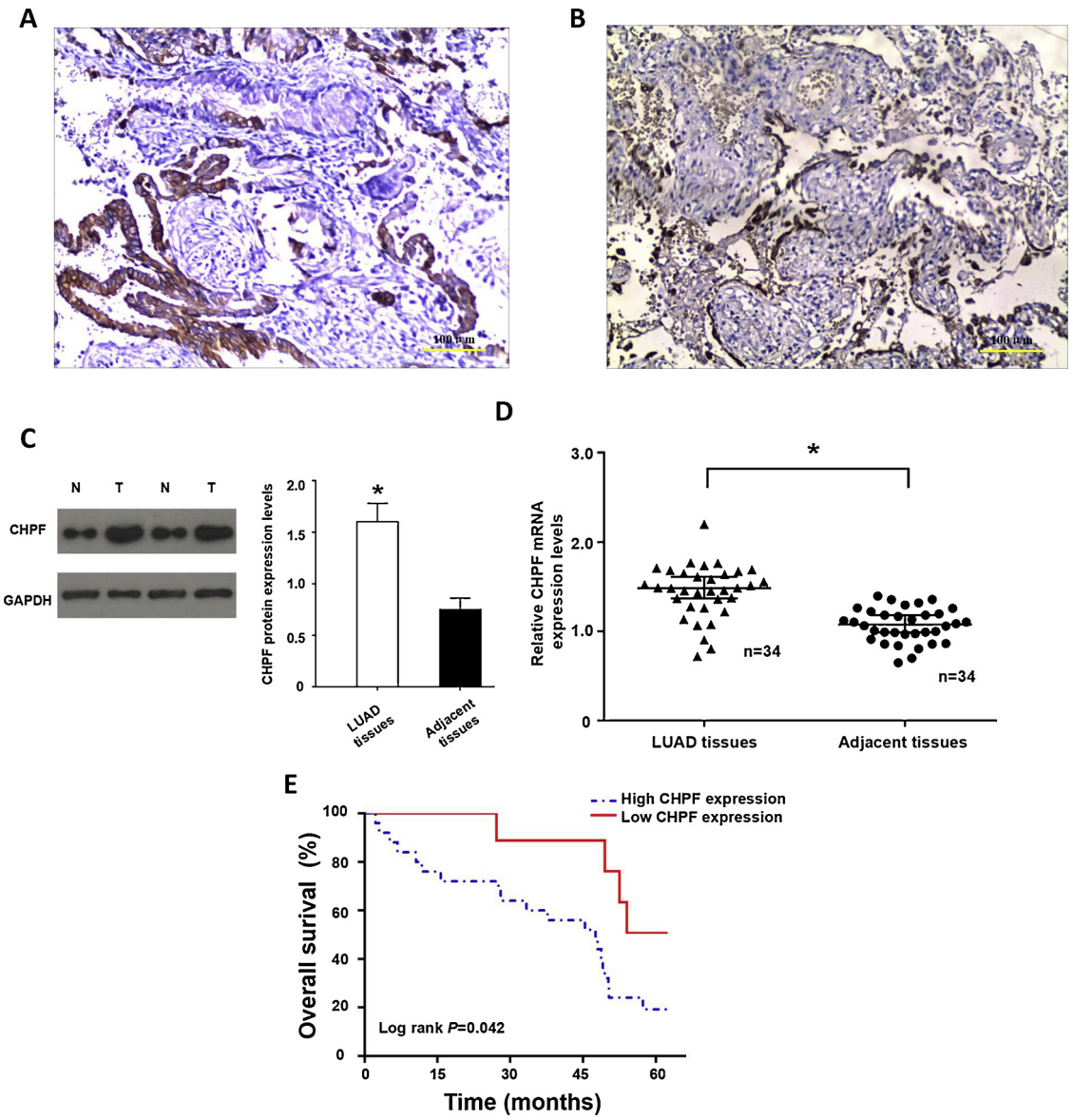

Fig. 2. The CHPF protein expression in cytoplasm of LUAD (A) and in cytoplasm of adjacent tissues (B) which was detected by IHC (×200). The expression levels of CHPF proteins in LUAD tissues and adjacent tissues using the western blotting (C). qPCR analysis of CHPF mRNA expression levels in LUAD tissues and adjacent tissues (D). Kaplan-Meier indicates OS curve of LUAD patients correlated with CHPF expression (E). Note: CHPF means chondroitin polymerizing factor; LUAD means lung adenocarcinoma; IHC means immunohistochemistry; N means adjacent tissues; T means LUAD tissues. * $P < 0.01$.

2.3. Immunohistochemistry (IHC) and assessment

A 4-μm thick section was cut from the paraffin-embedded tissue. Then, 3% H_2O_2 was used to block the endogenous peroxidase, followed by antigen retrieval by microwave heating. Afterwards, the sections were incubated with 10% normal goat serum and reacted with rabbit anti-human CHPF antibody (Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4 °C; the sections were later rinsed in phosphate buffered saline (PBS). Other slides were incubated with PBS overnight as the negative control. Finally, the sections were incubated with IgG as a secondary antibody at 37 °C. 3,3′-diaminobenzidine tetrahydrochloride (DAB) was used for visualization, then the sections were subsequently counterstained with hematoxylin.

The IHC sections were independently assessed by two pathologists. The percentage of positive tumor cells was semi-quantitatively graded as 0 (< 5%), 1 (5–25%), 2 (26–50%), and 3 (> 50%). The staining intensity of tumor cells was scored as 0 (negative staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). Both scores were multiplied to obtain the final immunoreactivity score. High expression of CHPF in tumor cells was defined as an immunoreactivity score of ≥ 4.

2.4. Lentiviral shRNA-mediated CHPF silencing

The shRNA interference target sequence was designed by using the CHPF gene (NM_024536) as a template according to the design principle of RNA interference sequences. The target sequence of shCHPF was 5′-CTGGCCATGCTACTCTTTG-3′. A well-defined non-specific control sequence (5′-TTCTCCGAACGTGTCACGT-3′) was used as a control group (shCtrl). The lentiviral vector system consisted of the GV115 vector, the pHelper 1.0 vector and the pHelper 2.0 vector (Genechem Co., LTD., Shanghai, China). Transfection was performed using the Genechem Transfection Reagent (Genechem Co., LTD.) according to the manufacturer's instructions, followed by additional incubation for 72 h. The cellular transfection efficiency was confirmed according to the percentage of GFP-positive cells under a fluorescence microscope.

Table 1
Correlations between CHPF expression and clinicopathological features in LUAD patients.

Variables	Cases (n = 34)	CHPF expression (%)		*P*-value
		Low (n = 11)	High (n = 23)	
Gender				0.914
Female	19	6 (31.6)	13 (68.4)	
Male	15	5 (33.3)	10 (66.7)	
Age (years)				0.549
≤60	13	5 (38.5)	8 (61.5)	
> 60	21	6 (28.6)	15 (71.4)	
Smoking				0.928
No	22	7 (31.8)	15 (68.2)	
Yes	12	4 (33.3)	8 (66.7)	
Differentiation				0.259
Well/moderate	11	5 (45.5)	6 (54.5)	
Poor	23	6 (26.1)	17 (73.9)	
Tumor size (cm)				0.017
≤3	12	7 (58.3)	5 (41.7)	
> 3	22	4 (18.2)	18 (81.8)	
LN metastasis				0.083
Negative	9	5 (55.6)	4 (44.4)	
Positive	25	6 (24.0)	19 (76.0)	
TNM stage				0.010
I/II	14	8 (57.1)	6 (42.9)	
III	20	3 (15)	17 (85)	
EGFR status				0.538
Wild	24	7 (29.2)	17 (70.8)	
Mutant	10	4 (40.0)	6 (60.0)	

Abbreviations: CHPF means chondroitin polymerizing factor; LUAD means Lung adenocarcinoma; LN, lymph node; EGFR, epidermal growth factor receptor.

Stable knockdown of CHPF in selected cells was confirmed using Real-time Quantitative Polymerase Chain Reaction Detecting System (qPCR) and western blotting.

2.5. RNA extraction and qPCR

First, total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was then quantified using a NanoDrop spectrophotometer (Thermo, Rockford, IL, MA, USA). RNA was reverse transcribed into cDNA by using M-MLV kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instruction. qPCR analysis was performed using a LightCycler 480 II real-time PCR instrument (Roche Applied Science, Indianapolis, IN, USA). The relative expression of CHPF was normalized to GAPDH, which was used as an internal reference. The primer sequence for CHPF was as follows: 5′-TGACTTCAACAGCGACACCCA-3′ (sense) and 5′-CACCCTGTTGCTG TAGCCAAA-3′ (antisense). The primer sequence of GAPDH was as follows: 5′-GGAACGCACGTACCAGGAG-3′ (sense) and 5′-CGGGATGGTG CTGGAATACC-3′ (antisense). The above primers were synthesized by GeneChem. The expression level of CHPF was calculated using the $2^{-\Delta\Delta CT}$ method.

2.6. Western blotting

Western blotting was used to analyze the protein levels. Cellular protein was extracted by using a lysis buffer. Equal amounts of cell lysate were separated on a 10% SDS-PAGE gel and transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked and incubated with the appropriate primary antibodies. The primary antibodies were as follows: rabbit anti-human CHPF antibody (Santa Cruz Biotechnology), anti-ERK, anti-phospho-ERK, anti-MAPK, anti-phospho-MAPK, anti-JNK and anti-phospho-JNK, which were all purchased from Abcam (Cambridge, MA, USA). Afterwards, the membranes were incubated with goat anti-rabbit IgG (1:1000; Bioworld Biotechnology CO., LTD., Nanjing, Jiangsu) secondary antibody. The results were normalized to the level of GAPDH (anti-GAPDH antibody (ab9485), Abcam). Finally, the band intensity was analyzed by optical density.

2.7. Cell proliferation by Celigo assay

Cells in the logarithmic growth phase were digested with trypsin (Sangon Biotech, Shanghai, China) and resuspended. Then, the cells were seeded into a 96-well plate at a density of 2000 cells/100 μL/well and incubated overnight at 37 °C containing 5% CO_2. From the second day after plating, the number of cells was continuously measured over 5 days using a Celigo image cytometer (Nexcelom Bioscience, Lawrence, MA, USA). Statistics were analyzed to plot the cell proliferation curve. The cell proliferation ratio was calculated based on the cell count and time point, and a growth curve was drawn based on the fold change in cell proliferation.

2.8. Colony formation assay

Cell clone formation was detected by colony analysis. Briefly, the cells were seeded into 6-well plates (10^3 cells/well) for 6 h and allowed to grow for 10 days into colonies by adding fresh medium. Finally, live colonies were visualized by 1% crystal violet staining and the number of colonies was calculated in each well.

2.9. Cell cycle analysis

The effect of CHPF on the cell cycle was analyzed by measuring the intracellular DNA content. In brief, cells were harvested and digested by trypsin, and rinsed twice with PBS. Then, cells were fixed with 75% ethanol for 1 h at 4 °C, washed with D-Hanks solution (Genechem Co., LTD.), and precipitated. Afterwards, they were incubated with 1 mg/mL RNase A at 37 °C for 20 min. The fixed cells were stained with 50 μg/mL propidium iodide (PI) (Cat. No. P4170, Sigma, St. Louis, MO, USA), followed by cell cycle analysis by Guava easy Cyte HT flow cytometer (Millipore, USA).

2.10. Cell apoptosis detection

Cell apoptosis was analyzed using the Annexin V-allophycocyanin kit (Annexin V-APC; Cat. No. 88-8007) (eBioscience, Thermo Fisher Scientific, USA) according to the manufacturer's instruction. Cells were collected and processed as described above. The cell concentration was adjusted to 1×10^6/mL by using a staining buffer. Finally, 100 μL of suspension was stained with 5 μL Annexin V-APC.

2.11. Xenograft experiment

The animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Lanzhou University, and all animal procedures were performed strictly according to the NIH guidelines for animal use. In brief, 6-week-old male BALB/C-Null nude mice (Cancer Institute of the Chinese Academy of Medical Science, Shanghai, China) were used for xenograft experiments. Each group consists of 6 BALB/C-Null nude mice. A total of 1×10^6 cells from shRNA-CHPF group and the shRNA-Ctrl group were inoculated subcutaneously into nude mice. After the injection, the volume of subcutaneous tumors was measured and calculated every 4 days in nude mice using the formula $V = 0.5 \times \text{length} \times \text{width}^2$. After 4 weeks, nude mice were sacrificed, and tumors were extracted for measurement and photography.

2.12. Statistical analysis

The data were expressed as mean ± SD. The relationship between CHPF expression and clinicopathological features of LUAD patients was analyzed by chi-square test. Comparisons between two groups were

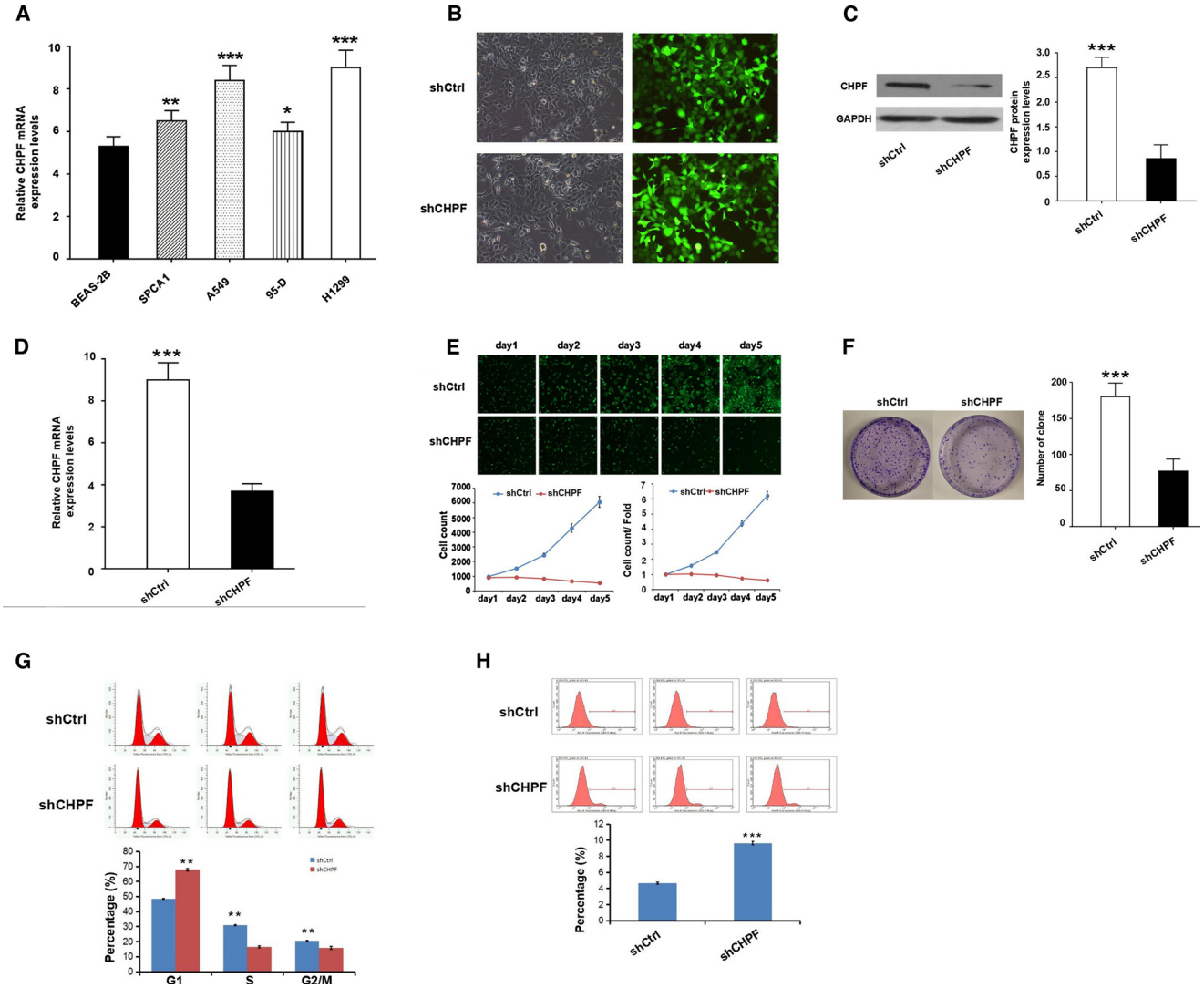

Fig. 3. (A) The expression levels of CHPF mRNA in LUAD cell lines and BEAS^{-2}B by using the qPCR. (B) Transfection efficiency was observed by fluorescence microscopy (100×). After transfection, the expression levels of CHPF protein and mRNA in LUAD cells line H1299 was assessed by western blotting (C) and qPCR (D). (E) Cell proliferation ability by Celigo assay. (F) Cell clone formation was detected through colony analysis. (G) Cell cycle of H1299 cell lines were examined through flow cytometry. (H) Analysis of apoptosis of H1299 cell lines using Annexin V-APC kit. Note: CHPF means chondroitin polymerizing factor; LUAD means lung adenocarcinoma; qPCR means real-time quantitative polymerase chain reaction; Annexin V-APC means Annexin V-allophycocyanin. Compared with BEAS^{-2}B, * $P > 0.05$; ** $P < 0.05$; *** $P < 0.01$.

performed by using the Student's *t* test. The Kaplan-Meier method was used to generate the survival curves of patients, and the Log-rank test was used to compare the survival rate of both groups. The primary outcome indicator was overall survival (OS). SPSS 19.0 (IBM, Armonk, NY, USA) software was used for statistical analysis, and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Bioinformatic results

To find the target genes in LUAD, we analyzed the differential gene expression in 57 pairs of samples based on TCGA. The volcano map revealed the changes in gene expression in LUAD samples and non-tumor samples (Fig. 1A). After careful screening, we found that CHPF (gene ID 79,924) was significantly upregulated in LUAD samples, compared to matched normal samples (Fig. 1B,C). Kaplan-Meier analysis demonstrated that the survival time of patients with high expression of CHPF was significantly shorter than those with low expression of CHPF (Fig. 1D).

3.2. The expression of CHPF in LUAD

To verify the above-described results from bioinformatic predictions, we examined the expression status of CHPF in 34 LUAD tissue samples. According to the results of IHC, the CHPF protein was mainly localized in the cytoplasm (Fig. 2A,B), whether it was a tumor or a normal cell. The expression of CHPF in LUAD tissues (23/34, 67.6%) was significantly higher than that in adjacent tissues (14/34, 41.2%; $P = 0.028$). In addition, western blotting and qPCR were used to quantitatively analyze the protein and mRNA expression of CHPF in LUAD and adjacent tissues. The protein and mRNA expression of CHPF were significantly higher in LUAD tissues compared to the adjacent tissues (Fig. 2C,D). These results confirmed the bioinformatic predictions.

According to the results of IHC, 34 patients with LUAD were divided into CHPF low expression group (n = 11) and CHPF high expression group (n = 23). We further analyzed the correlation between CHPF expression and the clinicopathological characteristics of patients with LUAD. As a result, the abnormal expression of CHPF was closely correlated with tumor size and TNM stage in LUAD patients (Table 1). In addition, these LUAD patients were followed-up. Similar to the results

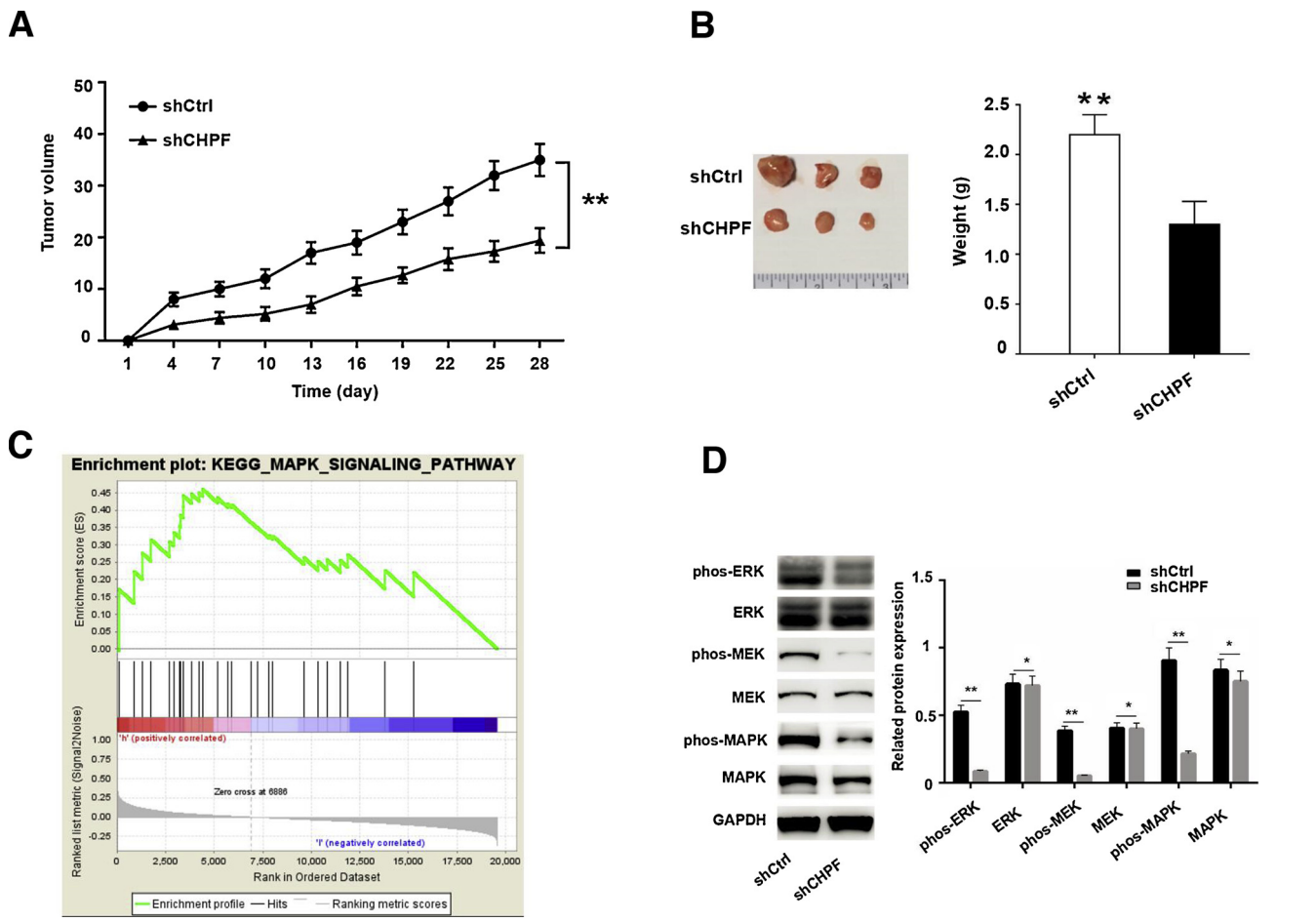

Fig. 4. (A) The subcutaneous tumor volume in nude mice was measured and calculated every four days. (B) The nude mice were sacrificed, and the tumors were taken out for measurement. (C) GSEA indicates that MAPK pathways have the strongest association with higher expression of CHPF. (D) CHPF regulates the MAPK pathway in LUAD. Note: CHPF means chondroitin polymerizing factor; KEGG means Kyoto Encyclopedia of Genes and Genomes; GSEA means Gene Set Enrichment Analysis. * $P > 0.05$; ** $P < 0.01$.

predicted by the Kaplan-Meier database, the survival time of LUAD patients with high expression of CHPF was significantly shortened compared to those with low expression (Fig. 2E).

3.3. CHPF enhances the proliferation of LUAD in vitro and in vivo

qPCR was used to detect the mRNA expression of CHPF in LUAD cell lines SPCA1, A549, 95-D, H1299, and normal lung epithelial cells BEAS-2B. Among them, the expression of CHPF was highest in H1299 cell line (Fig. 3A). To explore the function of CHPF in LUAD, lentiviral-mediated shRNA was utilized to silence CHPF expression in H1299 cells. After lentiviral infection for 72 h, a fluorescence microscope was used to observe H1299 cells, which revealed that the cell transfection efficiency was over 80% and the cells were normal (Fig. 3B). Western blotting and qPCR demonstrated that CHPF expression was significantly inhibited in shCHPF group compared to that in shCtrl group, which could be used for subsequent cell function assays (Fig. 3C,D).

To assess the effects of CHPF on the proliferation of LUAD cells, a Celigo image cytometer was used to record cell proliferation. Consequently, H1299 cells in the shCHPF group showed significant growth inhibition from day three compared to the shCtrl group. As the culture time increased, shCHPF exerted a more significant inhibition on cell proliferation (Fig. 3E). A colony formation assay was also one of the criteria for assessing tumor cell proliferation. The assay showed that the colony forming ability of H1299 cells in the shCtrl group was significantly higher than that in the shCHPF group (Fig. 3F). Compared with the shCtrl group, the number of cells was increased in the G1 phase and decreased in both the S and G2/M phases, suggesting that inhibition of CHPF expression could lead to G1 arrest of H1299 cells (Fig. 3G). The Annexin V-APC kit was used to analyze the effects of CHPF on apoptosis of H1299 cells. Compared with the shCtrl group, the number of apoptotic cells in the shCHPF group was increased, indicating that knockdown of CHPF significantly induced apoptosis in H1299 cells (Fig. 3H).

To determine whether CHPF knockdown affected LUAD tumor growth *in vivo*, H1299 cells from shCtrl and shCHPF groups were subcutaneously injected into BALB/C-Null nude mice to construct xenograft tumor growth models. After inoculation of H1299 cells, the volume of subcutaneous tumors in nude mice was measured and calculated every 4 days. Tumor volumes indicated that knockdown of CHPF expression inhibited the growth of subcutaneous tumors in nude mice but did not completely alter tumorigenesis (Fig. 4A). Tumors from the shCHPF group were significantly smaller compared with the shCtrl group (Fig. 4B). In conclusion, CHPF could promote the tumorigenicity of LUAD cells.

3.4. CHPF promotes tumor proliferation via the MAPK pathway

To explore the potential mechanism of CHPF on the proliferation of LUAD tumors, the TCGA samples were divided into CHPF high and low expression groups according to the median value, followed by GSEA. As shown in Fig. 4C, the KEGG MAPK signaling pathway was positively correlated with high CHPF expression. To further validate the GSEA results, we examined the expression of the MAPK signaling pathway-

associated proteins in both shCtrl and shCHPF groups. As a result, the expression of ERK, MEK, and MAPK of H1299 cells was not significantly different between both groups. However, compared with the shCtrl group, the expression of phosphorylated ERK, MEK, and MAPK in the shCHPF group was significantly decreased (Fig. 4D). The above-described results indicate that high expression of CHPF could activate the MAPK pathway *in vitro*, thereby promoting the proliferation and inhibiting apoptosis of LUAD cells.

4. Discussion

CHPF is the fifth important member found in the chondroitin sulfate polymerase family, which can interact with other family members to regulate the extension of the chondroitin sulfate chain [5,6]. The CHPF gene is located on human chromosome 2q35-q36 loci, containing a 236 bp 5′-untranslated region and a 2325 bp open reading frame [5]. Human CHPF consists of 775 amino acids with 3 potential N-glycosylation sites and type II transmembrane protein topology [5]. Kitagawa (2003) showed that CHPF is differentially expressed in human tissues, showing moderate expression in the brain, skeletal muscle, kidney, and liver, and high expression in the placenta and heart [5]. However, few studies have been carried out on the expression of CHPF in tumors. A study found that CHPF is highly expressed in human glioma tissues and 4 glioma cell lines, and confirmed that CHPF promotes growth and inhibits apoptosis in U251 glioma cells [3].

There has been no report relating the abnormal expression of CHPF to the progression of LUAD. In this study, bioinformatic methods were used to mine genes that were differentially expressed in LUAD. Among them, CHPF harbored a significant differential expression in LUAD. Meanwhile, we predicted that high CHPF expression indicated a worse prognosis. Based on this clue, we investigated the function of CHPF and its correlation with LUAD. The expression of CHPF in LUAD showed that it was differentially expressed in cancer tissues and adjacent tissues, confirming the results of previous biochemical analysis and matching the *in vitro* data. In addition, we analyzed the association of CHPF expression with clinicopathological characteristics of patients with LUAD. Consequently, high expression of CHPF was associated with tumor size and distant metastasis. Interestingly, it was not significantly associated with lymph node status, which may be due to the limited sample size. Therefore, we hypothesized that CHPF may affect the proliferation and apoptosis of LUAD cells.

Based on the above phenomena, we conducted *in vitro* cell studies to establish the function of CHPF in LUAD. Initially, we compared the expression of CHPF in several LUAD cell lines and normal lung epithelial cells BEAS^{-2}B, which showed that CHPF expression was upregulated in LUAD cell lines, consistent with the results from tissue expression. We further selected the LUAD cell line, H1299 for subsequent functional studies by inhibiting the expression of CHPF *via* lentiviral-mediated knockdown. Through a series of experiments, lentiviral-mediated CHPF knockdown could significantly inhibit the proliferation and tumorigenic ability of LUAD cells, while promoting cell apoptosis, leading to cell cycle arrest. In *in vivo* studies, a tumor xenograft model was established in nude mice. Consistent with the *in vitro* results, tumor size, volume, and weight were significantly reduced in the shCHPF group, validating that CHPF knockdown delayed tumor growth *in vivo*.

From tissue, cell, and animal data, we conclude that CHPF can promote tumor cell proliferation and anti-apoptosis, further leading to LUAD progression. The potential molecular mechanisms involved are yet to be elucidated. Through GSEA analysis, we found that CHPF is mainly enriched in the MAPK signaling pathway. MAPK are a group of serine-threonine protein kinases that can be activated by different extracellular stimuli, such as cytokines, neurotransmitters, hormones, cellular stress, and cell adhesion [7–10]. MAPK cascade activation is the center of various signaling pathways, playing a key role in diverse cell proliferation-related signaling pathways [11]. In unstimulated cells, MAPK is at rest [12]. After the cells are stimulated by growth factor or other factors, MAPK is activated-characterized by progressive phosphorylation. Therefore, we examined the expression and phosphorylation levels of MAPK signaling pathway-related molecules. As a result, CHPF is confirmed to promote the malignant behavior of LUAD cells by activating or partially activating the MAPK signaling pathway.

In this study, we confirmed that knockout of CHPF could significantly inhibit LUAD cell proliferation, cell cycle progression, and promote apoptosis. Our findings showed that CHPF was a carcinogenic factor of LUAD, which, therefore, could be used as a prognostic marker or therapeutic target.

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Conflict of interest

The authors report no conflicts of interest in this work.

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