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Involvement of CHP2 in the Development of Non–Small Cell Lung Cancer and Patients' Poor Prognosis

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Abstract: The present study aimed to investigate the expression levels and clinical significance of the calcineurin B homologous protein 2 (CHP2) in non–small cell lung cancer (NSCLC), and to study its effects on biological characteristics of NSCLC cells. Tumor and adjacent samples were collected from 196 NSCLC patients. Western blot analysis was used to detect the expression levels of the CHP2 in 8 pairs of NSCLC fresh tissues and 4 NSCLC cell lines. Immunohistochemical analysis was used to detect the expression of the CHP2 in 188 additional pairs of NSCLC wax block tissues. The data indicated that the expression levels of the CHP2 in the paraffin and fresh tissues of NSCLC were significantly higher than those of the adjacent tissues. According to the histo-score, univariate and multivariate analysis indicated that a high expression level of CHP2 was an important factor affecting the 5-year survival rate of NSCLC patients. After knocking down the expression of CHP2 in NSCLC cell lines, the proliferative, migratory, and invasive activities of NSCLC-CHP2 cells were decreased which were assessed by Western blotting, Cell Counting Kit-8, and transwell and wound-healing assays. In conclusion, the data demonstrated that CHP2 was highly expressed in NSCLC and that it could promote the development of NSCLC, suggesting its potential application for the therapy of NSCLC.

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Cancer is the second leading cause of death in the United States and worldwide.[1] The primary cause of cancer-related deaths is lung cancer, and this cancer type is considered the most common malignancy in the world.[2] Approximately 80% to 90% of lung cancers are non–small cell lung cancers (NSCLCs),[3,4] and in the clinic, the majority of the NSCLC patients present with locally advanced or metastatic disease.[5] The incidence of lung cancer is estimated to 1.8 million and the cancer-related deaths attributed to lung cancer causes are estimated to 1.6 million.[6] The recent application of targeted therapies has provided clinical benefits to patients with NSCLC.[6,7]

The development of lung cancer involves a variety of genetic and epigenetic changes that result in the conversion of normal cells into cancer cells. Calcineurin B homologous protein 2 (CHP2) is a member of the super family of N-myristolated EF-hand Ca^{2+}-binding proteins (CHPs).[8] CHPs play a significant role in the transmembrane Na^+/H^+ exchange[9–12] and contribute to the resistance to serum deprivation-induced cell death in malignant cells.[13] A recent study demonstrated that CHP2 promoted proliferation of breast cancer cells by activating the AKT signaling pathway and by transactivating FOXO3a expression.[14] It has been shown that the expression levels of CHP2 in leukemic cells are significantly increased, which may affect their proliferation.[15] An additional study highlighted that overexpression of CHP2 promoted proliferation of HEK293 cells, whereas suppression of CHP2 in HepG2 cells inhibited cell proliferation.[16,17] The enhanced expression levels of CHP2 in human OVCAR3 ovarian carcinoma cells contributed to an increased binding ability of the tumor cells to fibronectin.[18] Wallert and Provost and their research team have demonstrated that the expression of CHP2 was upward in tumor samples from patients with either adenocarcinoma or squamous cell carcinoma of the lung as well as in a broad range of NSCLC cell lines. In vitro experiments, reduce CHP2 gene expression in H1299 cells could decrease the ability of invasion and migration which the NHE1 played an essential role in this process.[19–27] These results indicated that CHP2 was implicated in the regulation of transmembrane Na^+/H^+ exchange and that it could promote

cancer proliferation. However, the clinical significance of CHP2 in NSCLC remain poorly understood.

In the present study, we investigated the expression levels of CHP2 in NSCLC clinical samples, as well as in experimental cell line models. The data revealed that CHP2 was upregulated in NSCLC tissues and cells, and that high CHP2 levels were negatively associated with the 5-year survival rate of the patients. The current study demonstrated for the first time that CHP2 acted as a novel tumor promoter of NSCLC and that it may serve as a new potential prognostic indicator and therapeutic target for this disease.

MATERIALS AND METHODS

Cell Lines and Cell Culture

The human NSCLC cell lines SPC-A-1, NCI-H1650, NCI-H1299, and A549, were purchased from the ScienCell (Carlsbad, CA). The cells were cultured in RPMI-1640 medium (Gibco), which contained 10% fetal bovine serum and antibiotics (100 U/mL penicillin, 0.1 mg/mL streptomycin). All cells were cultured in a 5% CO_2 atmosphere at 37°C.

Patient Information and Tissue Samples

A total of 196 pairs of NSCLC and adjacent nontumor tissues were obtained from NSCLC patients who received surgery at the Affiliated Hospital of Nantong University (188 pairs of tissues for immunohistochemical (IHC) analysis were collected from 2005 to 2011 and 8 pairs were collected in 2018 for Western blot from the Affiliated Hospital of Nantong University). Human surgical tissues were immediately frozen in liquid nitrogen and stored at −80°C. The patients had not received chemotherapy, radiotherapy, hormone therapy or other type of related antitumor therapies for at least a year before surgery. Cancer diagnosis was confirmed by histopathologic evaluation. The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hospital of Nantong University (Jiangsu, China). Written informed consent was provided by all participants.

Tissue Microarray (TMA) IHC Assay

NSCLC primary tumors and corresponding adjacent tissues were fixed in 10% formalin solution for 24 hours before being embedded in paraffin. TMA sections were then constructed by skilled pathologists in the Department of Clinical Pathology of our hospital using the UNITMA Quick-Ray manual staining system (UT06; UNITIA, Korea). Each paraffin block was cut into 4 μm sections. The qualified TMA slides were dewaxed and rehydrated. For antigen retrieval, slides were heated in sodium citrate buffer (10 mM, pH 6.0) for 3 minutes. After 3 washes of 0.01 M phosphate-buffered saline (PBS) (pH 7.4), the slides were then blocked by Bovine Serum Albumin (BSA; Sigma, St. Louis, MO) for 30 minutes, followed with incubation of primary antibody for CHP2 (1:400, EL913117-100; EterLife, UK) at 4°C overnight and secondary antibody (EnVision goat anti-rabbit HRP; Dako) for 1 hour. Finally, we used hematoxylin-eosin staining to confirm the quality of TMA sections. All slides were scanned and scored by the Automated Quantitative Pathology Imaging System (PerkinElmer) (the parameters and procedures were set by an experienced pathologist). The positive staining levels of the tumor cells were specified as follows: 0 (negative, blue), 1+ (weak, yellow), 2+ (positive, brownish red), and 3+ (strong positive, brown). Subsequently, a histo-score (H-score)[28–30] was used that was based on the staining strength and the percentage of positive cells. The H-score was used for counting the expression levels of CHP2. The average positive percentage was calculated and the following formula was applied: H-score = (% of weakly stained cells×1)+(% of positively stained cells×2) +(% of strongly positively stained cells×3). An H-score between 0 and 300 was obtained, wherein 300 equals 100% of the tumor cell staining corresponding to strongly positive (3+) staining. The X-tile software (Rimm Laboratory at Yale University; www.tissuearray.org/rimmlab) was used to select an appropriate cutoff value (here it is set to 170) and to partition the protein levels into 2 categories (no/low expression and high expression).

Western Blotting

NSCLC tissue samples were obtained from 8 NSCLC patients and matched with adjacent normal tissue samples for Western blot analysis. NSCLC cells were trypsinized and centrifuged. The pellets and tissue samples were resuspended in cell lysis buffer (P0013B, Beyotime Institute of Biotechnology) that contained 1% protease inhibitor (ST506; Beyotime Institute of Biotechnology) separately. Approximately 30 μg of proteins per lane were separated in 15% sodium dodecyl sulphate gels by polyacrylamide gel electrophoresis. The gels were transferred to polyvinylidene fluoride membranes (IPVH00010; Millipore Corporation), and the membranes were blocked with TBST containing 5% nonfat milk powder. Subsequently, the membranes were incubated with a primary antibody against CHP2 (1:500 dilution, GTX87522; Genetex) at 4°C overnight. Following washing with TBST, the membranes were incubated with secondary horseradish peroxidase–conjugated goat anti-rabbit IgG (1:2000, ab205718; Abcam) for 2 hours at room temperature. Immunoreactive proteins were evaluated using an enhanced chemiluminescence system (Bio-Rad, Hercules, CA). Glyceraldehyde 3-phosphate dehydrogenase (1:2000, AB0037; Abways) was used as the internal control sample. Signal intensities were subsequently quantified using the Image J quantification software.

Transfection of NSCLC Cell Lines

Predesigned shRNA duplexes were purchased from Biomics Biotechnologies Company. The sequences of *CHP2*-shRNA1, shRNA2, and shRNA3, were as follows: 5′-GGAGTTCACCAAGTCCTTA-3′, 5′-GAAGGAACAAACTTCACTA-3′, and 5′-CACTGGACAGGAATAAGAA-3′, respectively. A negative control duplex was also synthesized, of which the following sequence was used: 5′-TTCTCCGAACGTGTCACGT-3′. These recombinant plasmids were transfected into NSCLC cells using Lipofectamine 2000 (Thermo Scientific) and the cells were incubated in 6-well plates for 24 hours, according to the manufacturer's protocol.

Cell Proliferation Assay

Cell proliferation was measured by the Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology).

Untreated (parental) cells and cells treated with the empty vector (shControl) and/or the *CHP2*-shRNA inhibitor (sh*CHP2*) were incubated in 96-well plates. At 24, 48, 72, and 96 hours, 10 μL of CCK-8 solution was added into each well and incubated with the cells at 37°C for 2 hours. The absorbance was measured at 450 nm using a microplate spectrophotometer (Synergy HT; BioTek).

Wound-healing Assay

Wound-healing experiments were used to compare the migratory capacity of NSCLC control and transfected cells. The cells were grown in a monolayer to 100% confluence. Subsequently, the wounds were created by making a scratch using a 10 μL pipette tip. The cell monolayer was washed with PBS and cultured in a serum-free medium for 12 and 24 hours. At these time points, the wounds were photographed and the wound closure was measured using Image J software.

Transwell migration and invasion assays: Migration assays were conducted using 8 μm transwell chambers (353097; Falcon). A total of 3×10^5 cells were obtained in suspension in 100 μL of nonserum medium and seeded in the upper chamber of the transwell. The invasion assay was conducted by precoating of the upper surface inserts (BD Biosciences) with Matrigel 4 hours before cell seeding, as stated above. The lower chamber was filled with 600 μL of complete culture medium. The cells were incubated at 37°C. Following 24 hours of incubation, the transwells were washed with PBS and fixed in paraformaldehyde for 5 minutes. The upper chamber cells were cleaned with swabs and the lower surface of the membrane was stained with 0.1% crystal violet for 10 minutes. The infiltrating cells were viewed and counted in 5 randomly selected fields under a light microscope (Olympus). The method was performed as mentioned above to analyze the percentage of cell migration, with the exception of the Matrigel coating step being omitted.

Statistical Analysis

The measurement data were expressed as mean ± SD. A χ^2 test and a 1-way analysis of variance were used to test for group differences of CHP2 expression in cancer and adjacent normal tissues. A *t* test was used for data comparison between the 2 groups. All data were statistically analyzed using GraphPad Prism 6.01 (GraphPad) and SPSS 20.0 software (IBM). A *P*-value <0.05 was the cutoff for significant differences.

RESULTS

CHP2 is Overexpressed in Primary Human NSCLC Tissues

To determine whether CHP2 is upregulated in NSCLC, we applied IHC analysis on a TMA including 188 pairs of NSCLC tissues with their adjacent normal counterparts, and confirmed the upregulation of CHP2 expression in NSCLC ($P<0.001$) (Figs. 1A–F). The IHC results further revealed that CHP2 was mainly expressed in the cytoplasm and in the extracellular matrix (Figs. 1C1, C2, E1, E2). We further examined CHP2 expression levels in an additional dataset comprising 8 paired NSCLC tissues and their adjacent normal tissues by Western blot analysis. The data indicated that primary tumor tissues contained more CHP2 compared with the matched paracancerous tissues (Fig. 1G1). Statistical analysis (Fig. 1G2) indicated that CHP2 expression levels were significantly increased ($P<0.001$).

Association of CHP2 Expression With Clinicopathologic Parameters in NSCLC

To verify whether the elevated expression levels of CHP2 were involved in NSCLC progression, we explored the correlation between CHP2 expression and the clinicopathologic characteristics of the NSCLC patients. According to the H-score, the cohort was divided into high CHP2 expression group and low or no CHP2 expression group. The association between high expression levels of CHP2 and the clinicopathologic variables of the NSCLC patients was investigated (Table 1). A total of 126 of 188 subjects (67%) exhibited high expression of CHP2, whereas only 62 (33%) demonstrated low or no expression of CHP2. The Pearson χ^2 test indicated that high CHP2 expression levels were significantly associated with the clinical stage ($P=0.021$), tumor pathology ($P=0.041$), tumor differentiation ($P=0.049$), and tumor size ($P=0.047$). Concomitantly, no correlation was observed between CHP2 expression and other clinical parameters (eg, sex, age of diagnosis, smoking and lymph node metastasis) ($P>0.05$) (Table 1).

Impact of CHP2 Expression in Determining the Prognosis of NSCLC Patients

Univariate and multivariate analyses were conducted on NSCLC 5-year survival prognostic variables (Table 2). Univariate Cox regression analysis of all variables examined indicated that poor prognosis in patients with NSCLC was associated with overexpression of CHP2. In addition, the parameters smoking, clinical stage, tumor differentiation, and lymph node metastasis were significantly associated with the survival time of NSCLC patients. Multivariate Cox regression analysis conducted on the same group of NSCLC patients further confirmed that CHP2 overexpression was an independent prognostic factor for 5-year survival.

Survival Analysis

Kaplan-Meier survival curves confirmed that CHP2 expression negatively correlated with OS in NSCLC patients. This correlation was gradually decreased with increasing CHP2 expression levels (Fig. 2A). Moreover, the data indicated that NSCLC patients exhibited a negative association with the parameters smoking (Fig. 2B), poor differentiation (Fig. 2C), larger tumor diameter (Fig. 2D), lymph node metastasis (Fig. 2E), and late clinical stage (Fig. 2F).

Silencing of *CHP2* Reduces its Expression in NSCLC Cell Lines

The expression of the CHP2 was demonstrated in all 4 lines by Western blot analysis. The highest CHP2 levels were observed in H1299 and A549 cells, followed by the H1650 and SPC-A-1 cell lines (Fig. 3A). The first 2 types of cells expressed higher levels of CHP2 (H1299 and A549) compared with those of the H1650 and SPC-A-1 cell lines, and were transfected with 3 types of sh*CHP2* sequences (shRNA1, shRNA2, and

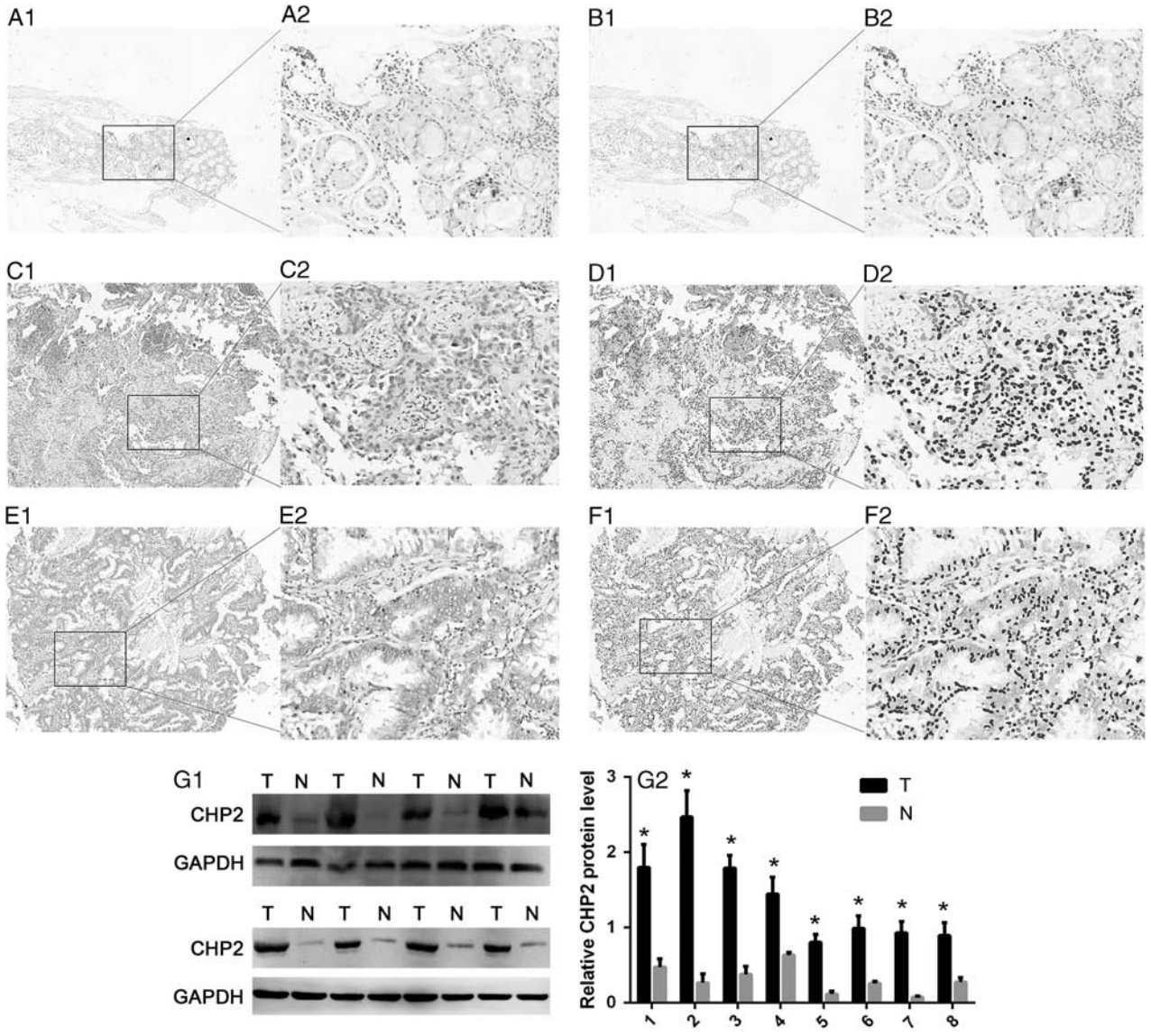

FIGURE 1. Tissue microarray immunohistochemical assay and Western blotting demonstrating the expression levels of CHP2 in non–small cell lung cancer tissues. Immunohistochemical staining of CHP2 was negative in adjacent normal lung tissues (A1, A2, B1, B2). CHP2 immunohistochemical staining was positive in squamous cell carcinoma tissues (C1, C2, D1, D2). CHP2 immunohistochemical staining was positive in lung adenocarcinoma tissues (E1, E2, F1, F2). CHP2 expression levels in lung cancer tissues were higher than those of the corresponding adjacent tissues (G1, G2); $*P < 0.01$ versus the negative control. The data are presented as mean ± SD. CHP2 indicates calcineurin B homologous protein 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

shRNA3) and with a negative control shRNA sequence. Western blot analysis was used to verify that CHP2 expression was decreased in sh*CHP2*-transfected cells (Fig. 3B). Moreover, shRNA1 exhibited the highest silencing efficiency, and it was therefore used in the following loss of function experiments.

Downregulation of *CHP2* reduces proliferation, migration and invasion of NSCLC cells in vitro. To investigate the effects of CHP2 on the biological ability of NSCLC cells, we initially tested their viability using the CCK-8 assay. NSCLC cell viability was significantly inhibited in H1299 (Fig. 4A1) and A549 cells (Fig. 4A2) transfected with shRNA1.

Concomitantly, a wound-healing experiment was conducted to evaluate the effects of *CHP2* knockdown on the migratory ability of NSCLC cells. The ability of shRNA1-transfected H1299 cells (Fig. 4B1) to migrate to blank areas was significantly delayed compared with that of the parental and shControl groups. Similarly, the migratory activity of A549 cells (Fig. 4B2) was further inhibited by *CHP2* deficiency.

Moreover, transwell assays were used to investigate the effects of *CHP2* downregulation on the migration and invasion of NSCLC cells. H1299 cells (Fig. 4D1) transfected with *CHP2* shRNA migrated at a much lower rate than that of the parental cells and the shControl group cells. The

TABLE 1. Correlation of Calcineurin B Homologous Protein 2 Expression in Tumorous Tissues With Clinicopathologic Characteristics in Non–Small Cell Lung Cancer Patients

		Calcineurin B Homologous Protein 2			
Clinicopathologic Parameters	**N**	**Low or No Expression**	**High Expression**	**Pearson χ^2**	***P***
Total	188	62	126		
Age at diagnosis (y)				2.464	0.116
<60	63	16	47		
≥ 60	125	46	79		
Sex				0.376	0.540
Male	73	26	47		
Female	115	36	79		
Smoking				0.032	0.857
No smoking	147	48	99		
Smoking	41	14	27		
Histopathology grading				6.380	0.041*
Adenocarcinoma	126	34	92		
Squamous cell carcinoma	45	21	24		
Adenosquamous carcinoma	17	7	10		
Differentiation				5.999	0.049*
Low grade	40	7	33		
Intermediate grade	125	45	80		
High grade	23	10	13		
Primary tumor				6.124	0.047*
T1	84	22	62		
T2	77	26	51		
T3+T4	27	14	13		
Lymph node metastasis				3.280	0.194
N0	116	34	82		
N1	37	12	25		
N2	35	16	19		
TNM stage				7.723	0.021*
I	104	30	74		
II	40	10	30		
III+IV	44	22	22		

*$P<0.05$.

experiment was repeated with the *CHP2*-shRNA transfected A549 cells (Fig. 4D2) and resulted in a similar result. As a result, the cells of the transfected group migrated to the lower chamber at a lower rate than that of the normal and control groups (Figs. 4C1, C2).

DISCUSSION

Lung cancer is a disease characterized by late-stage diagnosis and limited treatment options.[31] The development of personalized therapy (precise medicine) in the past decades has improved the therapeutic options of lung cancer.[32] With the successive determination of a series of lung carcinogenic genes, various studies conducted in China and worldwide have shown that targeted therapeutics greatly improve and prolong the prognosis and survival time of patients with NSCLC.[33–35] Future targeted therapy is the ultimate curative option for several patients with advanced NSCLC.[36,37] However, targeted therapy is not successful in certain patients due to intrinsic and

TABLE 2. Univariate and Multivariate Analyses of Predictive Factors for Prognosis of Non–Small Cell Lung Cancer Patients

	Univariate Analysis			Multivariate Analysis		
Characteristics	**HR**	***P***	**95% CI**	**HR**	***P***	**95% CI**
CHP2 expression (high vs. low)	2.300	<0.001*	1.441-3.672	3.049	<0.001*	1.811-5.132
Age of diagnosis (<60 vs. ≥ 60) (y)	0.780	0.228	0.520-1.168			
Sex (male vs. female)	1.409	0.104	0.932-2.132			
Smoking (no smoking vs. smoking)	1.562	0.046*	1.008-2.421	1.631	0.032*	1.044-2.549
Histopathology (grading Ad vs. Sq vs. ASC)	1.205	0.201	0.905-1.603			
Differentiation (low vs. middle vs. high grade)	1.880	<0.001*	1.339-2.638	1.524	0.018*	1.074-2.161
Primary tumor (T1 vs. T2 vs. T3+T4)	1.225	0.127	0.944-1.591			
Lymph node metastasis (N0 vs. N1 vs. N2)	1.445	0.001*	1.153-1.812			
TNM stage (I vs. II vs. III+IV)	1.338	0.004*	1.113-1.730			

Ad indicates adenocarcinoma; ASC, adenosquamous carcinoma; CHP2, calcineurin B homologous protein 2; CI, confidence interval; HR, hazard ratio; Sq, squamous cell carcinoma.
*$P<0.05$.

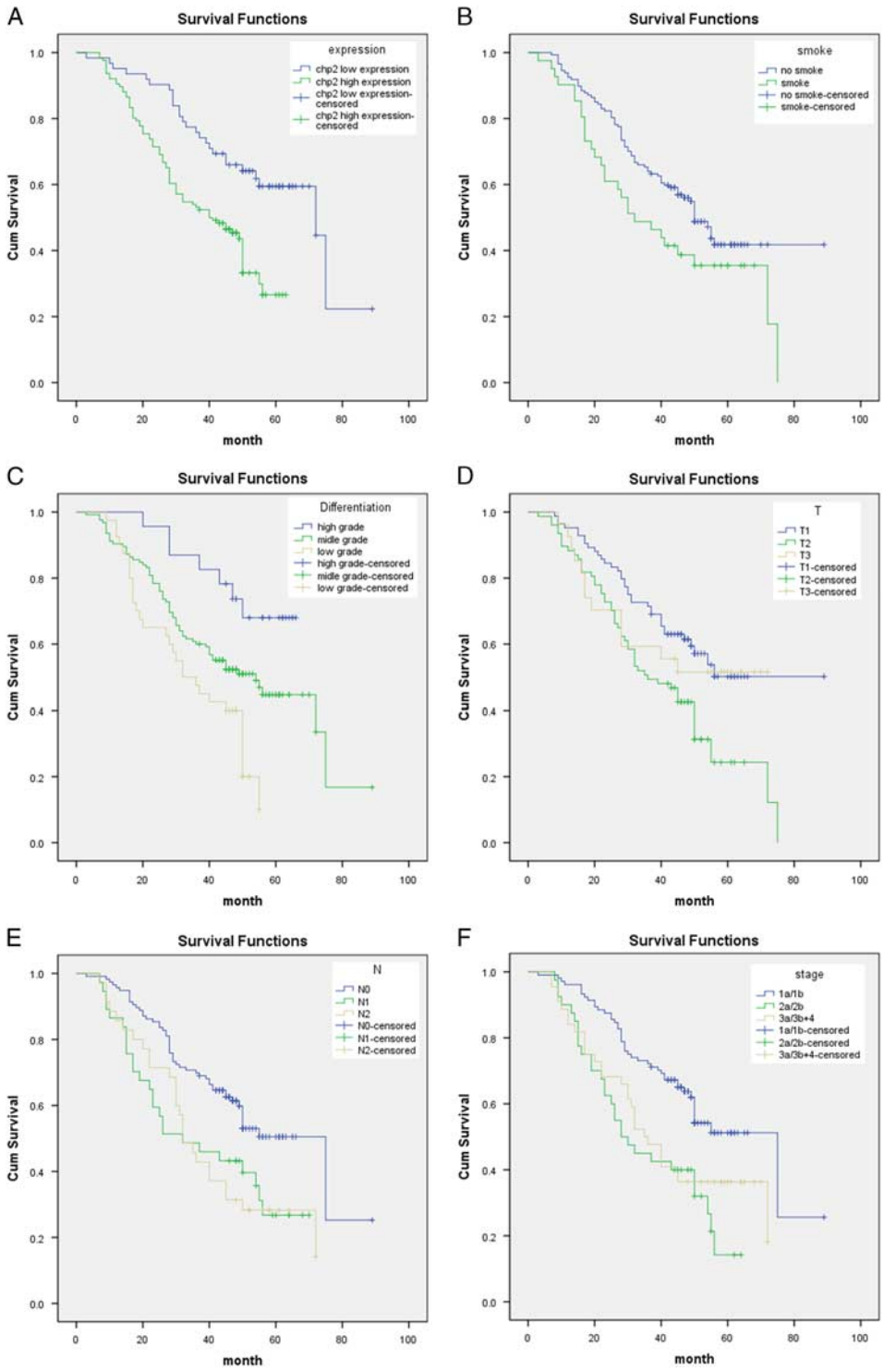

FIGURE 2. Kaplan-Meier survival curve analysis indicating the association of CHP2 expression with the 5-year survival rate of non–small cell lung cancer patients. In addition, other clinicopathologic parameters were also associated with the survival rate of the patients. Kaplan-Meier survival curve analysis indicated that the parameters CHP2 expression (A), smoking (B), tumor differentiation (C), primary tumor size (D), lymph node metastasis (E), and clinical stage (F) were significantly associated with overall survival in patients with non–small cell lung cancer. CHP2 indicates calcineurin B homologous protein 2.

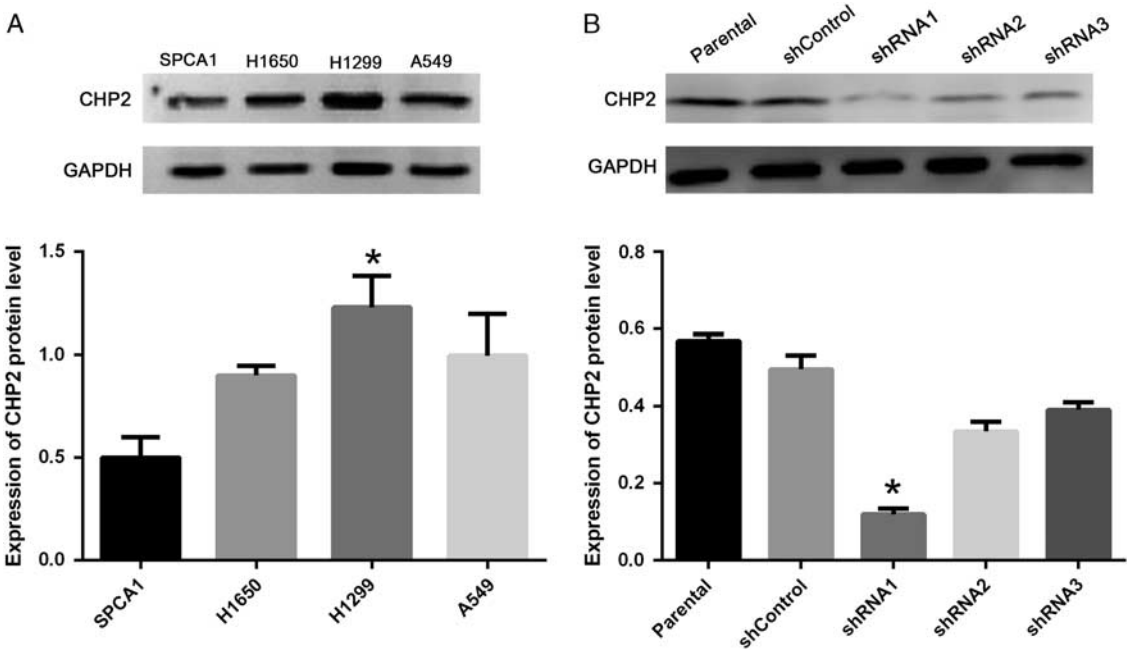

FIGURE 3. Detection of CHP2 expression in non–small cell lung cancer cell lines by Western blotting. A, CHP2 expression in 4 non–small cell lung cancer cell lines. B, Western blotting was used to detect the silencing effects of the 3 small interfering plasmids and the corresponding statistical quantograms. CHP2 indicates calcineurin B homologous protein 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. *$P < 0.01$ versus the negative control. The data are presented as the mean ± SD.

acquired drug resistance and due to the negative effect of driver genes.[38] Therefore, the identification of novel molecular targets can be used in the development of new therapies for lung cancer.

CHP2 is one member of the super family of N-myristoylated, EF-hand Ca^{2+}-binding proteins (CHPs), which has been studied in several types of human cancer, and exerts a diverse range of biological effects on tumor cells and other tissues. For example, the expression levels of CHP2 in leukemic primary cells were significantly increased, and may play a critical role in leukemic cell growth.[15,39] In addition, CHP2 expression levels were significantly increased in human OVCAR3 ovarian carcinoma cells and contributed to an increased binding ability of the tumor cells to fibronectin.[14] The present study provided IHC data demonstrating that CHP2 was highly expressed in NSCLC tissues compared with the expression levels noted in adjacent tissues, which is consistent with these previous reports. This was confirmed by experiments conducted in tissues and cells.

The samples were further analyzed by univariate Cox regression analysis with regard to all variables, and the results indicated a significant correlation between survival time in patients with NSCLC and overexpression of CHP2 (hazard ratio = 2.300, 95% confidence interval: 1.441-3.672, $P < 0.001$). Multivariate Cox regression analysis of the same group of NSCLC patients further indicated that CHP2 overexpression (hazard ratio = 3.049, 95% confidence interval: 1.811-5.132, $P < 0.001$) was an independent risk factor for the 5-year survival of patients with NSCLC. Kaplan-Meier survival curves demonstrated that the upregulation of CHP2 correlated significantly with poor prognosis of NSCLC patients suggesting that CHP2 overexpression may be used as an independent prognostic factor of NSCLC. Therefore, the data suggest that CHP2 can promote the deterioration of NSCLC.

Metastasis is a characteristic trait of malignant tumors. Several oncogenic drivers are responsible for the malignant transformation, invasion, and metastasis of the tumors. It has been shown that the ectopic expression of CHP2 promotes the proliferation of HEK293 cells.[16,17] A recent report confirmed that the overexpression of CHP2 promoted the proliferation and tumorigenicity of breast and ovarian cancers.[14,18] The present study demonstrated by CCK-8, wound healing and transwell assays that *CHP2* shRNA knockdown inhibited NSCLC cell growth, invasive and migratory activities. It has been shown that CHP2 is an oncogenic protein that affects the biological characteristics of NSCLC.

The specific mechanisms of cancer formation and cancer progression remain unclear. Previous studies have shown that CHP2 is mainly expressed in the cytoplasm and plasma membrane and that it contains a nuclear export sequence at the C-terminal region.[10,13,18,40] It has been shown that CHP2 is a putative signaling molecule that couples local Ca^{2+} concentration changes to a variety of specific signal-response cascades.[8,41,42] This function is noted in other EF-associated Ca^{2+}-binding proteins. However, a recent study that examined CHP2 expression in patient specimens by IHC analysis reported nuclear positive staining.[14] Although these hypotheses require further investigation, the current study provides evidence by IHC data supporting the cytoplasmic and cell membrane expression of CHP2. The present study suggested that CHP2 could promote the development of NSCLC.

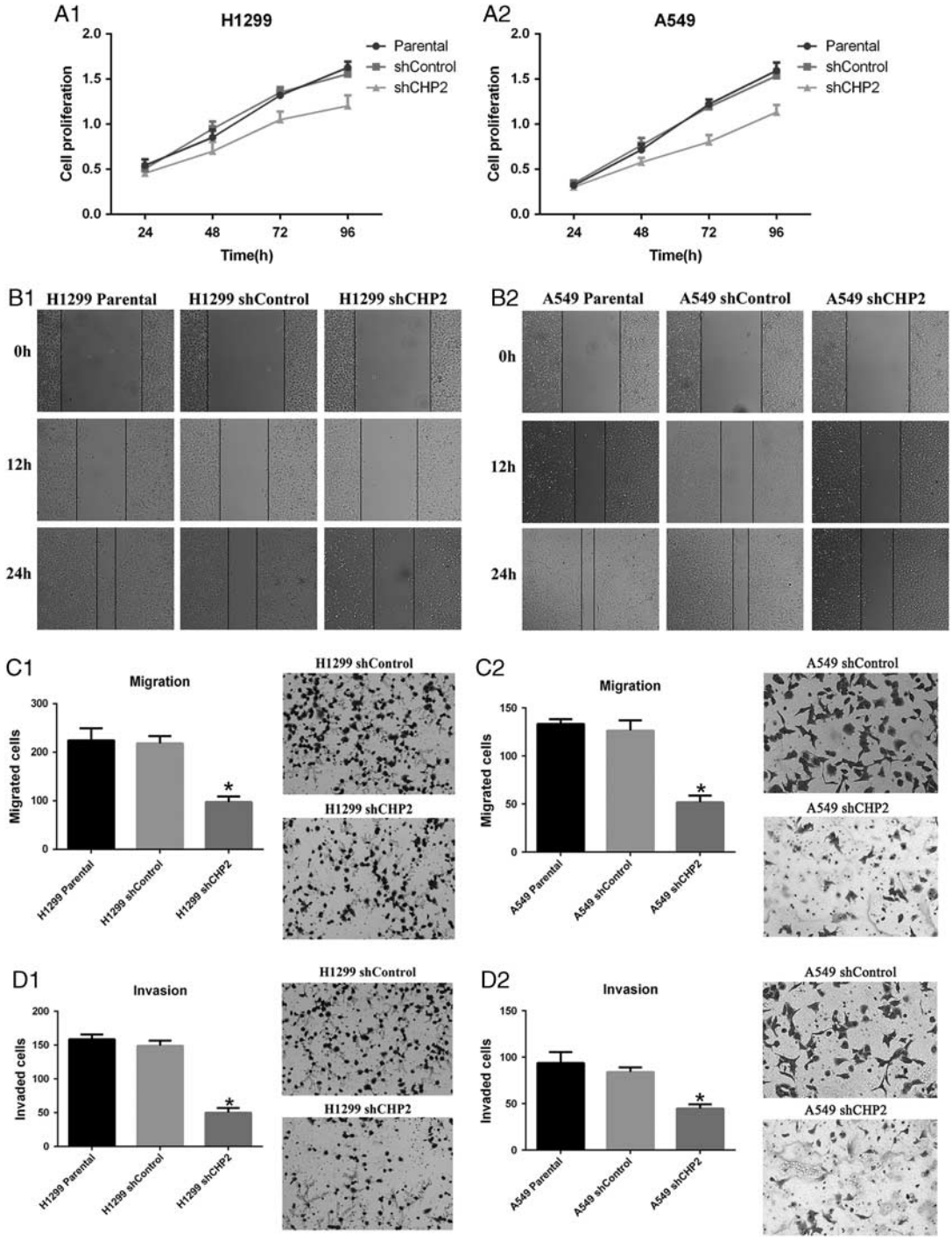

FIGURE 4. CHP2-mediated regulation of non–small cell lung cancer cell proliferation, migration and invasion in vitro. The CCK-8 assay was used to detect the effects of CHP2 on H1299 and A549 cell viability (A1, A2). The H1299 and A549 cell lines were categorized into 3 groups as follows: blank control (parental) group, negative control (shControl) group and *CHP2* interference (sh*CHP2*) group. The CCK-8 assay was performed to detect the effects of knocking out *CHP2* on the viability of non–small cell lung cancer cells. The wound-healing assay was used to detect the effects of CHP2 on the migratory ability of H1299 and A549 cells (B1, B2). H1299 and A549 tumor cells were obtained and classified into the aforementioned groups. The cell distances were recorded at 0, 12, and 24 hours, respectively, and photographed with a microscope. Transwell assays were used to examine the effects of CHP2 on H1299 (C1, C2, D1, D2) and A549 cell migration (C1, C2) and invasion (D1, D2). H1299 and A549 tumor cells were classified into the aforementioned groups and 5 fields were randomly selected from each chamber. The number of cells was counted in each field and plotted as a histogram (left panels). Typical images from microscopical assessment of the shControl and the sh*CHP2* groups (right panels) are shown (magnification, ×200). *$P < 0.01$ versus the negative control. The data are presented as the mean ± SD. CCK-8 indicates Cell Counting Kit-8; CHP2, calcineurin B homologous protein 2.

In conclusion, the data demonstrated that CHP2 was markedly overexpressed in NSCLC cells and in tumor tissues and that it may act as an oncogenic protein in NSCLC promoting NSCLC tumorigenesis and progression. Therefore, it is worthwhile to assess the molecular diagnostic ability of CHP2 in NSCLC and its further use as a promising therapeutic target.

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