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# Chloride Intracellular Channel 1 Expression Is Associated With Poor Prognosis of Lung Adenocarcinoma

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**Abstract.** *Background/Aim: Chloride intracellular channel 1 (CLIC1) is a member of the chloride channel protein family. The aim of this study was to clarify the role of CLIC1 in lung adenocarcinoma. Patients and Methods: The expression levels of CLIC1 in 74 patients with completely resected lung adenocarcinoma were analyzed by immunohistochemistry. Overall survival was assessed in relation to the expression level of CLIC1. Moreover, in the lung cancer cell lines A549 and PC9, CLIC1 expression was inhibited by small interfering RNA. The function of CLIC1 was analyzed in these cell lines. Results: High expression of CLIC1 was associated with short overall survival compared to low expression (p=0.0327). Multivariate analysis revealed that CLIC1 expression was an independent prognostic factor. Knockdown of CLIC1 inhibited cell proliferation and migration through suppression of the p38 MAPK signaling pathway in A549 and PC9 cells. Conclusion: CLIC1 may be a useful prognostic factor in lung adenocarcinoma.*

Chloride channels have been implicated in the regulation of electrical excitability, interepithelial fluid transport, ion homeostasis, pH levels, cell volume, and the cell cycle (1). Therefore, chloride channels may be involved in the process of tumorigenesis. Chloride intracellular channel 1 (CLIC1) is a member of the chloride channel protein family. CLIC1 is widely found in a variety of tissues, mostly in the cytoplasm. However, CLIC1 can switch from a soluble cytoplasmic conformation to a transmembrane isoform (2). Regarding CLIC1 expression in cancer, CLIC1 protein levels are increased in gastric cancer (3), colorectal cancer (4), and hepatocellular carcinoma (5). For lung adenocarcinoma, CLIC1 expression has been shown to correlate with prognosis after surgery (6). Moreover, in A549 cells, CLIC1 was shown to be an important regulator of $Ca^{2+}$ signaling that controls cancer cell survival (7). However, little is known about the effects of CLIC1 on lung cancer cell function. Therefore, the aim of this study was to clarify the clinical importance and role of CLIC1 using spiral arrays of postoperative lung cancer specimens and lung adenocarcinoma cell lines.

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*Key Words:* Chloride intracellular channel 1, immunohistochemistry, lung adenocarcinoma, spiral array.

## Patients and Methods

*Patients.* We analyzed 74 consecutive patients who underwent complete surgical resection of adenocarcinoma at Kobe University Hospital between January 2014 and December 2014. Pathological staging was determined based on the seventh edition of the TNM classification for lung cancer (8). The methods of data collection and analysis were approved by the institutional review board (permission number: 160117), and written informed consent was obtained from all patients.

*Spiral array.* The method of constructing the spiral array block has been described in detail previously (9).

Briefly, the tissue sample block was sent to Pathology Institute Corporation (Toyama, Japan). Then, 50- to 100-μm-thick slices of the sample block were cut and rolled up into cylindrical reels. One of the reels was embedded vertically in the recipient block to create a spiral array block. Finally, 4.0-μm sections were created for histopathological analysis.

*Immunohistochemistry.* Immunohistochemistry was performed using a standard procedure previously reported (10). Briefly, tissue sections from spiral array blocks were deparaffinized in limonene and

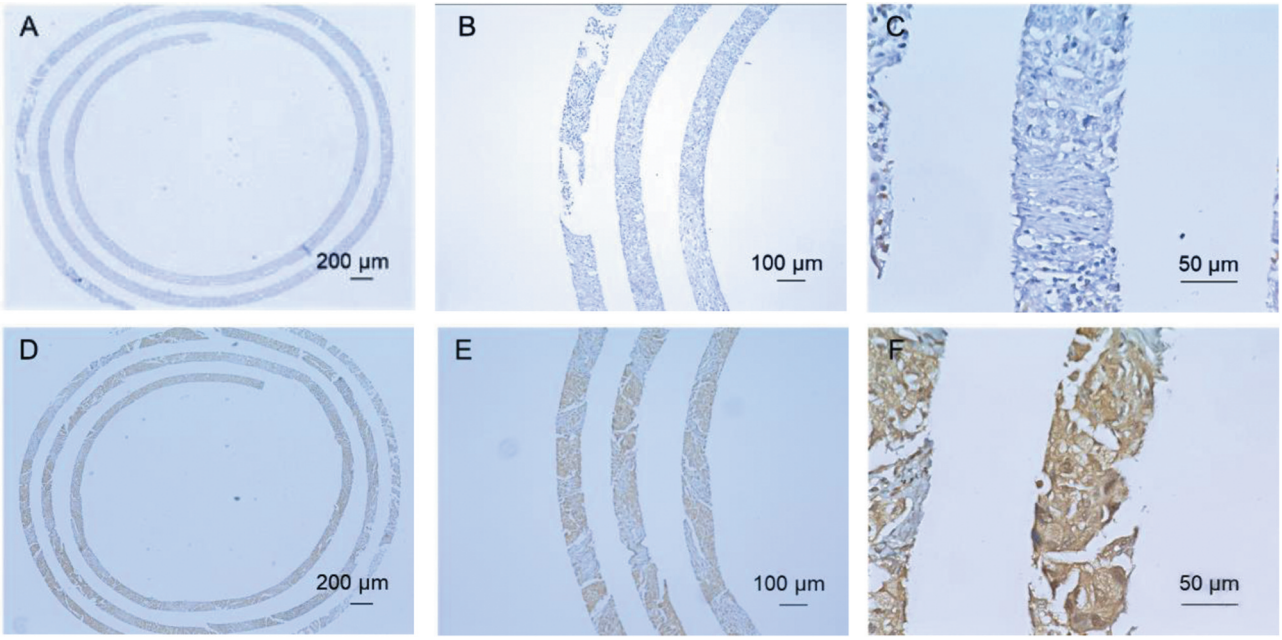


Figure 1. *The upper images show typical negative staining for CLIC1 of the spiral array specimens (A, B, C). The lower images show typical positive staining for CLIC1 (D, E, F).*

dehydrated in a graded alcohol series. For activation of antigen by heating, the slides were heated for 20 min at 121˚C in 10 μM citrate buffer (pH 6.0) in an autoclave. Endogenous peroxidase was blocked with 3% hydrogen peroxide in absolute methyl alcohol. Then, the slides were blocked in 2.5% horse serum for 60 min at room temperature. Next, a primary antibody against CLIC1 (Proteintech, IL, USA) was applied to slides at 4˚C overnight (1:200). The slides were incubated with ImmPRESS Reagent (Vector Laboratories, CA, USA) for 30 min. Finally, DAB staining was performed using ImmPACT DAB reagent (Vector Laboratories). High expression was defined as a case in which the number of positive cells increased by 50% or more compared to those of normal tissue (Figure 1).

*Cell culture*. The lung adenocarcinoma cell lines A549 and PC9, human large cell lung cancer cell line H460, human lung squamous cell carcinoma cell line H520, and human small cell lung cancer cell lines DMS53 and DMS114 were purchased from ATCC (Manassas, VA, USA). The lung adenocarcinoma cell line II18 was purchased from RIKEN Cell Bank (Ibaraki, Japan). These cells were cultured at 37˚C with 5% CO-1640 medium (Wako, Osaka, Japan) containing 10% fetal bovine serum and 1% penicillin–streptomycin (Nacalai Tesque, Kyoto, Japan).

*CLIC1 knockdown by small interfering RNAs*. CLIC1 siRNAs (#1: s635 and #2: s636) and negative control siRNA (#4390843) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Knockdown was performed according to the manufacturers' protocol. Briefly, cultured cells were confirmed to be 60-80% confluent in 6-well plates. The siRNA or control siRNA was mixed with Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific) in Opti-MEM medium (Thermo Fisher Scientific), and the mixture was then added to the cells. The cells were collected and analyzed 24 or 48 h after knockdown.

*Reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR*. Total cellular RNA extraction and RT-PCR were performed as previously reported (11). Extraction of total cellular RNA was performed using Sepasol-RNA I Super G (Nacalai Tesque) according to the manufacturer's protocol. Complementary DNA was generated using SuperScript III First-Strand Synthesis SuperMix for real-time PCR (Thermo Fisher Scientific). qRT-PCR was performed using a Thermal Cycler Dice Real Time System II (TaKaRa, Shiga, Japan). Relative mRNA levels were calculated with the ΔΔCt method using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control. The primers used in this study were as follows: 5'-ACCGCAGGTCGAATTGTTC-3' and 5'-ACGGTGGTAACATTGAAGGTG-3' for CLIC1 and 5'-GCACC GTCAAGGCTGAGAAC-3' and 5'-ATGGTGGTGAAGACGCC ATG-3' for GAPDH.

*Cell proliferation assay*. A549 and PC9 cells in the logarithmic growth phase were seeded in 96-well plates at a density of 5,000 cells/well and precultured in a 5% $CO_2$ incubator for 24 h. CLIC1 siRNA and negative control siRNA were transfected into these cells, and the cells were cultured at 37˚C in a 5% $CO_2$ incubator. Then, a total of 10 μl of Cell Counting Kit-8 (CCK-8) solution (Dojindo, Kumamoto, Japan) was added to each well, and the cells were incubated for another 120 min. Absorbance was measured at 450 nm using a spectrophotometer (Bio-Rad, iMark microplate reader, CA, USA).

*Scratch assay*. A scratch assay was performed as described previously (12). A549 and PC9 cells were seeded in 6-well plates.

Table I. *Patient characteristics (n=74).*

| CLIC1 expression | High (n=19) | Low (n=55) | *p*-Value |
|---|---|---|---|
| Male | 10 | 31 | 0.26 |
| Age, yr | 67±8.4 | 72±7.9 | |
| Smoking history | 11 | 33 | 1 |
| Lobectomy | 16 | 40 | 0.372 |
| Partial resection | 3 | 15 | |
| <pT2 | 9 | 27 | 1 |
| ≥pT2 | 10 | 28 | 1 |
| pN0 | 15 | 44 | 1 |
| ≥pN1 | 4 | 11 | |
| pM0 | 19 | 55 | |
| Pleural invasion | 6 | 21 | 0.785 |
| Lymphatic invasion | 7 | 15 | 0.385 |
| Vascular invasion | 10 | 19 | 0.183 |
| Pathological stage | | | |
| IA | 8 | 28 | 0.599 |
| ≥IB | 11 | 27 | |
| R0 | 19 | 55 | |
| EGFR mutation | 5 | 11 | 0.538 |
| Adjuvant chemotherapy | 9 | 14 | 0.09 |

Data are shown as the number or mean±SD. CLIC1: Chloride intracellular channel 1; EGFR: epidermal growth factor receptor.

When the cells grew to 100% confluency after knockdown of CLIC1, a monolayer was scratched using a p200 pipette. Images were taken under a BZ-X700 fluorescence microscope (Keyence, Osaka, Japan) at different times according to the growth speed of the cells. The wound width was analyzed by ImageJ (13).

*Western blotting*. A protocol for western blotting has been described previously (14). The amount of protein used in this study was standardized at 20 μg. Primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA): β-actin (#4967), p38 MAPK (#9212), p-p38 MAPK (#9215), cleaved caspase 3 (#9661), BAX (#2772) and Bcl2 (#4223). The primary antibody for CLIC1 was the same antibody used for immunohistochemical staining.

*Statistical analysis*. Fisher's exact test or Pearson $\chi^2$ tests were used for between-group comparisons of the categorical variables. For the univariate analysis, cumulative survival was estimated by the Kaplan–Meier method, and differences were assessed by the log-rank test. A multivariate regression analysis was conducted according to the Cox proportional hazard model. All *p*-values reported are 2-sided, and *p*-values less than 0.05 were considered significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for the R software program (The R Foundation for Statistical Computing, version 2.13.0) (15). More precisely, it is a modified version of the R commander (version 1.27) that includes statistical functions that are frequently used in biostatistics.

## Results

CLIC1 was expressed in the cytosol of cells as detected by immunohistochemistry (Figure 1). The clinical characteristics of the 74 patients classified by CLIC1 expression are summarized in Table I. The number of patients with high and low CLIC1 expression was 19 and 55, respectively. There were no significant differences in clinical parameters between the two groups.

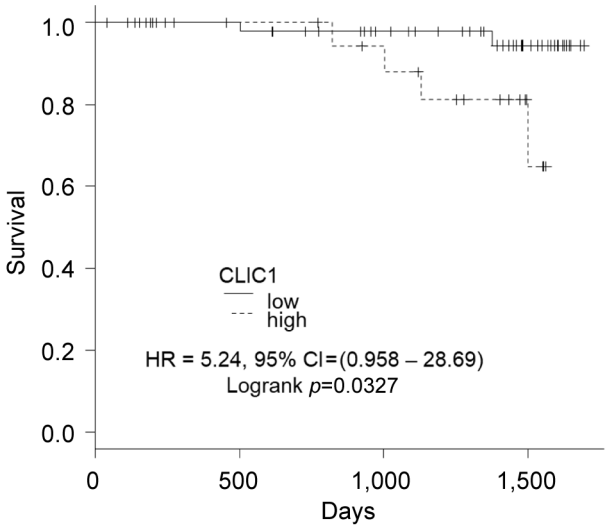


Figure 2. *Kaplan–Meier curve showing short overall survival in patients with high CLIC1 expression (p=0.0327).*

Table II. *Univariate analysis of CLIC1 and clinical parameters for overall survival.*

| Variables | Cutoff | MST | 95%CI | p-Value |
|---|---|---|---|---|
| CLIC1 | Low | NA | NA-NA | 0.0327 |
| | High | NA | 1502-NA | |
| pT factor | <2 | NA | NA-NA | 0.286 |
| | ≥2 | NA | NA-NA | |
| pN factor | 0 | NA | NA-NA | 0.0826 |
| | ≥1 | NA | 1131-NA | |
| Pleural invasion | Negative | NA | NA-NA | 0.237 |
| | Positive | NA | NA-NA | |
| Lymphatic invasion | Negative | NA | NA-NA | 0.585 |
| | Positive | NA | 1502-NA | |
| Vascular invasion | Negative | NA | NA-NA | 0.0874 |
| | Positive | NA | NA-NA | |

CLIC1: Chloride intracellular channel 1; CI: confidence interval; MST: median survival time; NA: not available; p: pathological.

The Kaplan–Meier curve of overall survival is shown in Figure 2. CLIC1 expression was significantly correlated with poor prognosis ($p$=0.0327). The results of univariate and multivariate analyses are shown in Table II and Table III, respectively. High expression of CLIC1 was found to be an independent prognostic factor for poor prognosis.

Table III. *Multivariate analysis of CLIC1 and clinical parameters for overall survival.*

| Variables | Cutoff | HR | 95%CI | *p*-Value |
|---|---|---|---|---|
| CLIC1 | Low *vs.* high | 10.6 | 1.12-100 | 0.0395 |
| pT factor | <2 *vs.* ≥2 | 1.25 | 0.1-15.5 | 0.862 |
| pN factor | 0 *vs.* ≥1 | 5.7 | 0.65-49.9 | 0.116 |
| Pleural invasion | Negative *vs.* positive | 1.86 | 0.17-30.2 | 0.658 |
| Lymphatic invasion | Negative *vs.* positive | 0.65 | 0.01-4.27 | 0.653 |
| Vascular invasion | Negative *vs.* positive | 1.04 | 0.09-11.1 | 0.977 |

CI: Confidence interval; CLIC1: chloride intracellular channel 1; HR: hazard ratio.

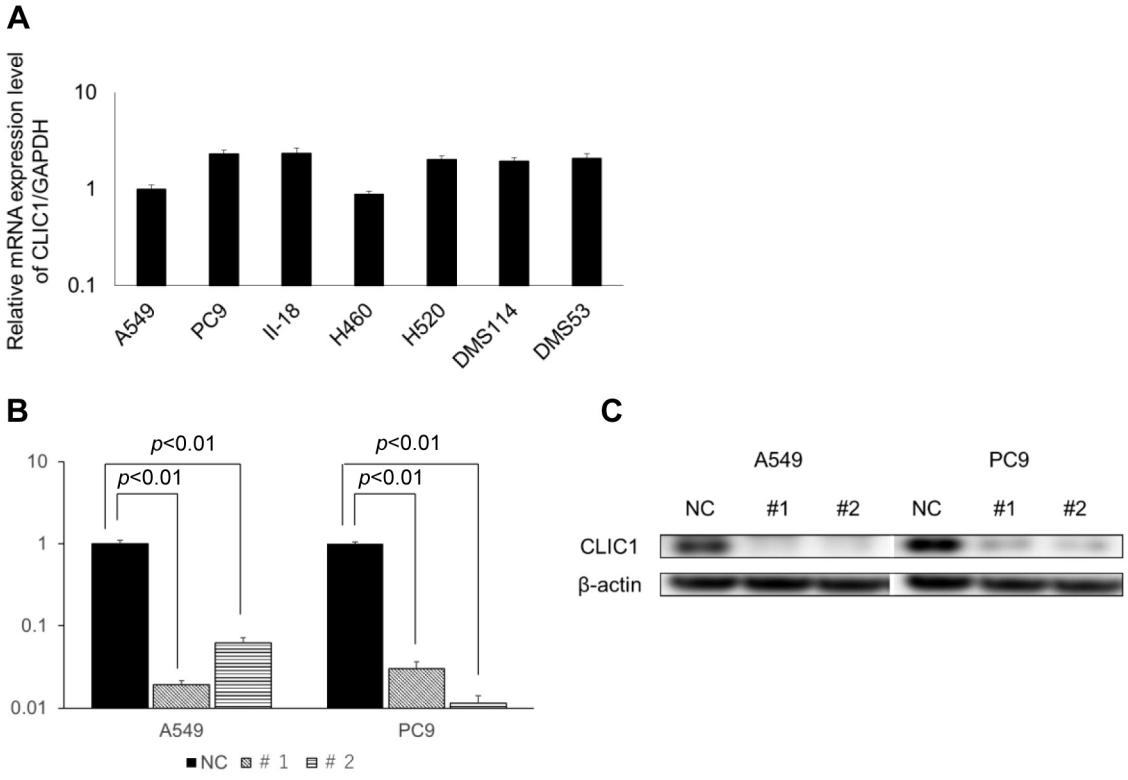


Figure 3. *Expression of CLIC1 was knocked down by CLIC1 mRNA. A) Relative mRNA expression of CLIC1 in the indicated lung cancer cell lines. B) Real-time PCR and C) western blotting showing the knockdown effect of CLIC1 siRNA (#1 and #2). NC: Negative control.*

To confirm the expression of CLIC1 in various lung cancer cell lines, we evaluated the mRNA levels of CLIC1 by real-time PCR. A549 cells, which have been shown to express CLIC1 in the Human Protein Atlas, were used as the standard (Figure 3) (16). These results suggested that CLIC1 was expressed in various types of lung cancer cell lines. A549 and PC9 cells were transfected with CLIC1 siRNA or negative control siRNA. Twenty-four hours after transfection, knockdown efficiency was assessed by real-time PCR. CLIC1 expression was significantly down-regulated by both siRNA #1 and #2 of CLIC1 compared to the negative control in A549 and PC9 cells ($p<0.01$) (Figure 3B). The protein expression level of CLIC1, which was evaluated by western blotting, was similarly suppressed (Figure 3C).

Next, cell proliferation was evaluated using CCK-8 assays. In A549 cells, siRNA #1 significantly inhibited cell proliferation ($p<0.05$). However, siRNA #2 inhibited cell proliferation with a significant difference in PC9 cells ($p<0.05$) (Figure 4).

To confirm the effect of CLIC1 on cell migration, we performed a scratch assay. Compared to the negative control, CLIC1 knockdown suppressed cell migration in PC9 cells

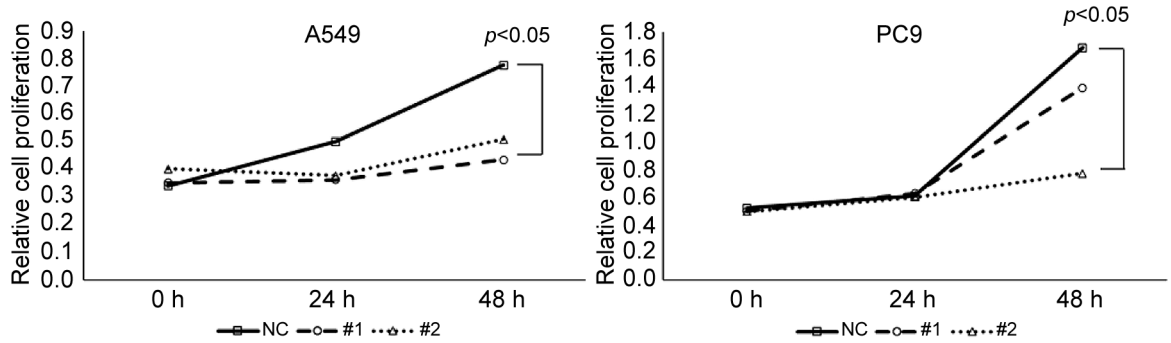


Figure 4. *CCK-8 assay showing that cell proliferation was suppressed in cells with knockdown of CLIC1 via CLIC1 siRNA (#1 and #2).*

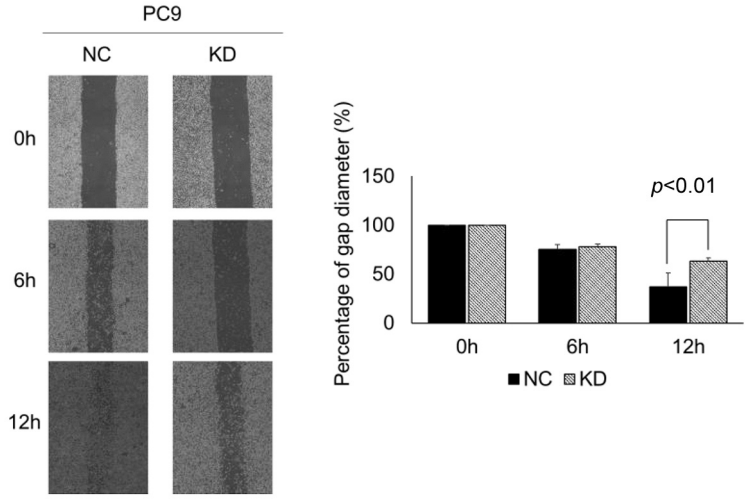


Figure 5. *Scratch assay showing cell migration was suppressed in PC9 cells in which CLIC1 was knocked down by CLIC1 siRNA. NC: Negative control; KD: knockdown.*

(Figure 5). These results suggested that CLIC1 could regulate the migration of lung adenocarcinoma cells.

Western blotting was performed to confirm the effect of CLIC1 on signal transduction. We evaluated the protein expression levels of CLIC1, β-actin, p38, and p-p38 (Figure 6A). CLIC1 knockdown increased the protein expression of p-p38 in both A549 and PC9 cells. For the protein expression levels of CLIC1, β-actin, cleaved caspase 3, BAX, and Bcl-2, increased protein expression of cleaved caspase 3 was observed only in A549 cells after siRNA-mediated knockdown of CLIC1 (Figure 6B). These results suggested that CLIC1 regulated cell proliferation by regulating apoptosis through the p38 MAPK signaling pathways in A549 cells.

## Discussion

In this study, we demonstrated that CLIC1 was an independent prognostic factor in lung adenocarcinoma. Regarding its molecular mechanism, CLIC1 was shown to play an important role in the regulation of cell proliferation, migration and the MAPK pathway.

Immunohistochemistry analysis of postoperative lung adenocarcinoma specimens showed that high expression of CLIC1 was associated with poor prognosis, which is consistent with a previous report (6). Moreover, in the TCGA database, high CLIC1 mRNA expression was also associated with poor prognosis (16). In contrast, a study evaluating the gene expression of ion channels in lung adenocarcinoma revealed that the CLIC3, CLIC4, and CLIC5 levels were down-regulated, while CLIC6 expression was upregulated in tumor cells (17). Thus, even ion channels belonging to the same CLIC family were shown to be differentially expressed in tumor cells.

In the present study, we showed that CLIC1 regulated cell proliferation by regulating apoptosis through the p38 MAPK signaling pathways in A549 cells but not in PC9 cells. A previous report showed that p38 induced apoptosis in breast

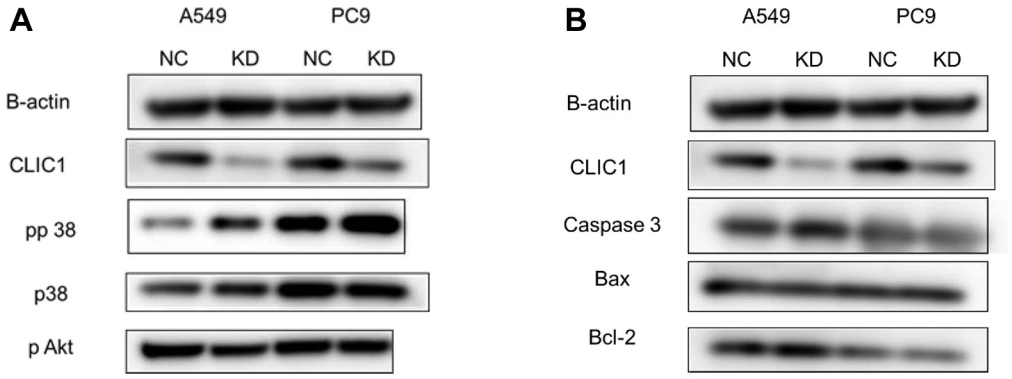


Figure 6. *Expression of p-p38 and cleaved caspase 3 were increased in CLIC1 knocked down A549 cells. And only p-p38 was increased in CLIC1 knocked down PC9 cells. A) Western blotting showing an increase in the protein expression of p-p38 in both A549 and PC9 cells and B) cleaved caspase 3 in A549 cells with siRNA-mediated knockdown of CLIC1. NC: Negative control.*

cancer cells expressing wild-type p53 (18). Since A549 cells express wild-type p53 and PC9 cells express mutated p53 (19), cell apoptosis was observed only in A549 cells.

The limitations of the present study are the small sample size and number of deaths. Therefore, prognostic factors such as T or N factors were not associated with poor prognosis.

In conclusion, CLIC1 was found to be an independent prognostic factor in lung adenocarcinoma. Moreover, CLIC1 may play an important role in the progression of lung adenocarcinoma. Therefore, in the future, CLIC1 might be a therapeutic target for lung cancer.

## Conflicts of Interest

The Authors declare no conflicts of interest associated with this manuscript.

## Authors' Contributions

YY and TN wrote the manuscript. YY and RS performed immunostaining. TK and MY made the database. TN and NJ performed pathological analysis. YM collected the samples. KK and DH provided technical advice. YY and YN conducted the statistical analysis. All Authors analyzed the data, conceived the study, and read and approved the final manuscript.

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## References

1 Peretti M, Angelini M, Savalli N, Florio T, Yuspa SH and Mazzanti M: Chloride channels in cancer: Focus on chloride intracellular channel 1 and 4 (CLIC1 AND CLIC4) proteins in tumor development and as novel therapeutic targets. Biochim Biophys Acta *1848(10 Pt B)*: 2523-2531, 2015. PMID: 25546839. DOI: 10.1016/j.bbamem.2014.12.012

2 Argenzio E and Moolenaar WH: Emerging biological roles of Cl- intracellular channel proteins. J Cell Sci *129(22)*: 4165-4174, 2016. PMID: 27852828. DOI: 10.1242/jcs.189795

3 Chen CD, Wang CS, Huang YH, Chien KY, Liang Y, Chen WJ and Lin KH: Overexpression of CLIC1 in human gastric carcinoma and its clinicopathological significance. Proteomics *7(1)*: 155-167, 2007. PMID: 17154271. DOI: 10.1002/pmic.200600663

4 Petrova DT, Asif AR, Armstrong VW, Dimova I, Toshev S, Yaramov N, Oellerich M and Toncheva D: Expression of chloride intracellular channel protein 1 (CLIC1) and tumor protein D52 (TPD52) as potential biomarkers for colorectal cancer. Clin Biochem *41(14-15)*: 1224-1236, 2008. PMID: 18710659. DOI: 10.1016/j.clinbiochem.2008.07.012

5 Wei X, Li J, Xie H, Wang H, Wang J, Zhang X, Zhuang R, Lu D, Ling Q, Zhou L, Xu X and Zheng S: Chloride intracellular channel 1 participates in migration and invasion of hepatocellular carcinoma by targeting maspin. J Gastroenterol Hepatol *30(1)*: 208-216, 2015. PMID: 24989236. DOI: 10.1111/jgh.12668

6 Wang W, Xu X, Wang W, Shao W, Li L, Yin W, Xiu L, Mo M, Zhao J, He Q and He J: The expression and clinical significance of CLIC1 and HSP27 in lung adenocarcinoma. Tumour Biol *32(6)*: 1199-1208, 2011. PMID: 21858536. DOI: 10.1007/s13277-011-0223-0

7 Lee JR, Lee JY, Kim HJ, Hahn MJ, Kang JS and Cho H: The inhibition of chloride intracellular channel 1 enhances $Ca^{2+}$ and reactive oxygen species signaling in A549 human lung cancer cells. Exp Mol Med *51(7)*: 1-11, 2019. PMID: 31316050. DOI: 10.1038/s12276-019-0279-2

8 Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, Postmus PE, Rusch V, Sobin L, International Association for the Study of Lung Cancer International Staging Committee and Participating Institutions: The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol *2(8)*: 706-714, 2007. PMID: 17762336. DOI: 10.1097/JTO.0b013e31812f3c1a

9 Fukuoka J, Hofer MD, Hori T, Tanaka T, Ishizawa S, Nomoto K, Saito M, Uemura T and Chirieac LR: Spiral array: a new high-throughput technology covers tissue heterogeneity. Arch Pathol Lab Med *136(11)*: 1377-1384, 2012. PMID: 23106583. DOI: 10.5858/arpa.2011-0393-OA

10 Ito T, Ishii G, Nagai K, Nagano T, Kojika M, Murata Y, Atsumi N, Nishiwaki Y, Miyazaki E, Kumamoto T and Ochiai A: Low podoplanin expression of tumor cells predicts poor prognosis in pathological stage IB squamous cell carcinoma of the lung, tissue microarray analysis of 136 patients using 24 antibodies. Lung Cancer *63(3)*: 418-424, 2009. PMID: 18657337. DOI: 10.1016/j.lungcan.2008.06.008

11 Nagano T, Edamatsu H, Kobayashi K, Takenaka N, Yamamoto M, Sasaki N, Nishimura Y and Kataoka T: Phospholipase cε, an effector of ras and rap small GTPases, is required for airway inflammatory response in a mouse model of bronchial asthma. PLoS One *9(9)*: e108373, 2014. PMID: 25269075. DOI: 10.1371/journal.pone.0108373

12 Liang CC, Park AY and Guan JL: In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc *2(2)*: 329-333, 2007. PMID: 17406593. DOI: 10.1038/nprot.2007.30

13 Schneider CA, Rasband WS and Eliceiri KW: NIH Image to ImageJ: 25 years of image analysis. Nat Methods *9(7)*: 671-675, 2012. PMID: 22930834. DOI: 10.1038/nmeth.2089

14 Hatakeyama Y, Kobayashi K, Nagano T, Tamura D, Yamamoto M, Tachihara M, Kotani Y and Nishimura Y: Synergistic effects of pemetrexed and amrubicin in non-small cell lung cancer cell lines: Potential for combination therapy. Cancer Lett *343(1)*: 74-79, 2014. PMID: 24139969. DOI: 10.1016/j.canlet.2013.09.019

15 Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant *48(3)*: 452-458, 2013. PMID: 23208313. DOI: 10.1038/bmt.2012.244

16 The Human Protein Atlas. Available at: https://www.proteinatlas.org/ [Last accessed on November 14, 2021]

17 Ko JH, Gu W, Lim I, Bang H, Ko EA and Zhou T: Ion channel gene expression in lung adenocarcinoma: potential role in prognosis and diagnosis. PLoS One *9(1)*: e86569, 2014. PMID: 24466154. DOI: 10.1371/journal.pone.0086569

18 Chen L, Mayer JA, Krisko TI, Speers CW, Wang T, Hilsenbeck SG and Brown PH: Inhibition of the p38 kinase suppresses the proliferation of human ER-negative breast cancer cells. Cancer Res *69(23)*: 8853-8861, 2009. PMID: 19920204. DOI: 10.1158/0008-5472.CAN-09-1636

19 Chou CW, Lin CH, Hsiao TH, Lo CC, Hsieh CY, Huang CC and Sher YP: Therapeutic effects of statins against lung adenocarcinoma *via* p53 mutant-mediated apoptosis. Sci Rep *9(1)*: 20403, 2019. PMID: 31892709. DOI: 10.1038/s41598-019-56532-6

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