NCAPH is negatively associated with Mcl-1 in non-small cell lung cancer

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Received February 27, 2020; Accepted June 25, 2020

DOI: 10.3892/mmr.2020.11359

Abstract. Lung cancer has a high mortality rate worldwide. Non-SMC condensin I complex subunit H (NCAPH) has been identified to be one of the regulatory subunits of the condensin I complex, which is essential for the correct packaging and segregation of chromosomes in eukaryotes. NCAPH is abnormally overexpressed in various types of cancer. A pro-survival member of the Bcl-2 family, myeloid cell leukemia sequence 1 (Mcl-1) is also frequently overexpressed in multiple cancers and is associated with poorer clinical outcomes for patients. The association of NCAPH and Mcl-1 proteins with the clinical and pathological features of non-small cell lung cancer (NSCLC) remains to be elucidated. In the current study, the positive percentage of NCAPH in the non-cancerous lung tissues was revealed to be higher compared with that in NSCLC. However, the positive percentage of Mcl-1 in the non-cancerous lung tissues was lower compared with NSCLC. In addition, NCAPH high-expression patients had a higher overall survival rate compared with patients exhibiting low expression, whereas the Mcl-1 high-expression group had a lower survival rate. Pairwise association in 260 cases of NSCLC revealed that overexpression of the NCAPH protein was negatively associated with Mcl-1 expression and vice versa. The results of multivariate Cox proportional hazard regression analysis also indicated that NCAPH and Mcl-1 demonstrated potential as distinct prognostic factors that may be used in NSCLC. The expression of NCAPH and Mcl-1 may be associated with, and act as distinct molecular marks for the prediction of a poor prognosis in patients with NSCLC.

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Key words: non-small cell lung cancer, non-SMC condensin I complex subunit H, myeloid cell leukemia sequence 1, prognosis

Introduction

Lung cancer has been widely acknowledged to have the highest mortality of all cancer types worldwide, and is classified pathologically as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (1). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are identified as the most common subtypes of NSCLC (2). Surgery is the most general treatment for early-stage NSCLC, and the combination of cytotoxic and platinum drugs has become the standard for NSCLC chemotherapy (3). However, the long-term survival for postoperative patients with NSCLC remains poor. Previous studies have indicated that the five-year survival rates of patients with NSCLC with stage Ia, Ib, IIa and IIb are 70, 60, 55 and 40%, respectively (4,5). Postoperative adjuvant chemotherapy has a limited effect on improving the survival rate of patients with NSCLC (6). The biomarker cluster of differentiation 24, has been recently identified for use in the prediction of NSCLC disease-free survival (7). However, there are other promising biomarkers with confirmed prognostic value in NSCLC, including matrix metallopeptidase 9, aurora kinase A and enhancer of zeste 2 polycomb repressive complex 2 subunit (8).

Non-SMC condensin I complex subunit H (NCAPH) is a member of the condensin I complex, which is a recently identified superfamily of proteins termed kleisins (9). Condensin I complex consists of three non-SMC subunits; NCAPH,

chromosome-associated protein (CAP)D2 and CAPG (10). Condensin serves an indispensable role in chromosome-wide gene regulation and therefore controls the architecture and segregation of sister chromatids (11). In the presence of type I topoisomerase, the condensin complex may introduce positive supercoils into relaxed DNA, while type II topoisomerase is involved in nicked DNA transformation into a positive knotted form (12). Biallelic mutations in CAPD2, NCAPH or CAPD3 are essential to ensure accurate mitotic chromosome condensation in neuron stem cells, ultimately affecting neuron pool and cortex size (13). The authors of the present study previously demonstrated that NCAPH is highly expressed in colon cancer, the knockdown of which inhibits cell proliferation, migration, and xenograft tumor formation abilities (14). However, patients with colon cancer and the overexpression of NCAPH have a markedly improved prognosis compared with patients who demonstrate low-expression of NCAPH (14). Other studies note that highly expressed condensin I complex in non-SMC is associated with the progression of multiple human cancers: Ryu *et al* (15) demonstrated that NCAPH is expressed at a higher level in metastatic melanoma cell lines compared with less aggressive primary tumor cell lines. Other studies have revealed that NCAPH can serve as a potential prognostic indicator for hepatocellular carcinoma and nasopharyngeal cancer (16).

A number of antigens, including carbohydrate antigen 125, are commonly used clinically as auxiliary indicators for the diagnosis of lung cancer, but they exhibit low sensitivity (17). Myeloid cell leukemia sequence 1 (Mcl-1), which is a member of the Bcl-2 family, was originally considered to be an effective short-term promoter for cell survival during the differentiation of bone marrow cells (18-21). Studies have demonstrated that Mcl-1 is a pro-survival protein that is overexpressed in human malignant tumors (22,23). Mcl-1, which is involved in chemotherapy resistance and metastasis, is often highly expressed in NSCLC and its association with other markers can improve diagnostic sensitivity (24). The intrinsic apoptotic signaling pathway is closely associated with NCAPH (GO:0097193: http://www.coexpedia.org/) (25). The dual expression of NCAPH and Mcl-1 proteins, and their association with clinical and pathological features in NSCLC remain unknown, with the exception of the aforementioned studies.

In the present study, the expression of NCAPH and Mcl-1 was determined in 260 cases of NSCLC, and the association of NCAPH and Mcl-1 expression with clinical and pathological characteristics of NSCLC, and its prognosis, was discussed.

Materials and methods

Clinical data. Paraffin-embedded NSCLC tissues from 260 patients diagnosed with primary NSCLC, none of whom had received any treatment before the surgery. All patients had complete clinical and follow-up data. (194 males and 66 females, mean age 60.1±12.76), and 52 control non-cancerous lung disease sections (37 males and 15 females, mean age 57.45±14.56), including bronchiectasis and pneumatocele, were collected from the Second Xiangya Hospital between January 2010 and December 2016. The present study was approved by the Scientific and Research Ethics Committee of the Second Xiangya Hospital (approval no. S039/2011).

Table I. Data sources of the mutation patterns of NCAPH and Mcl-1 in various types of cancer.

Author, year	Cancer type	(Refs.)
Taylor *et al* (2018)	Stomach adenocarcinoma	(30)
Jordan *et al* (2017)	Non-small cell lung cancer	(31)
Janjigian *et al* (2018)	Esophagogastric carcinoma	(32)
Soumerai *et al* (2018)	Endometrial cancer	(33)
Witkiewicz *et al* (2015)	Pancreatic cancer	(34)
Ghandi *et al* (2019)	Cancer of unknown primary	(35)
Yaeger *et al* (2018)	Colorectal cancer	(36)
Pereira *et al* (2016)	Breast cancer	(37)

NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1.

Patients were fully informed about specimen usage and data retrieval prior to the acquisition of specimens. No information or images that may expose any relevant patient identification or violate any individual rights are presented in the present study. Written informed consent was obtained from each patient. Informed consent was obtained from a parent and/or legal guardian for subjects <18 years old. All patients with NSCLC underwent surgery and none received neoadjuvant radiotherapy or chemotherapy. Histological diagnosis (26) and staging (27) were performed for all patients to confirm NSCLC. Tissue microarray technology was performed as previously described (28,29).

Immunohistochemistry (IHC) staining and scores. IHC was performed to detect the expression and cellular location of NCAPH and Mcl-1. IHC assay was performed as previously described (16,28). Tissues were fixed in paraformaldehyde dehydrated in a graded alcohol series, embedded in paraffin and sectioned at 3 μm. Sections were then deparaffinized, rehydrated and endogenous peroxidase inactivated using methanol containing 0.3% H_2O_2. The slides were incubated with appropriate pre-immune serum (Normal Goat Serum, Beyotime Institute of Biotechnology, cat. no. C0269; diluted to 10% in PBS+0.1% Tween20) for 30 min at room temperature to eliminate nonspecific staining, and the sections were stained overnight at 4˚C with 1:100 dilution of primary antibody to NCAPH (Rabbit-anti-NCAPH antibody, Fine Test; Wuhan Fine Biotech Co., Ltd., cat. no. FNab05579), recombinant anti-Mcl-1 antibody (Abcam, cat. no. ab32087). Slides were then exposed to an appropriate secondary antibody (MaxvisionTM2 HRP-Polymer anti-Mouse/Rabbit IHC kit, Fuzhou Maixin Biotech. Co., Ltd., dilution: 1:1, cat. no. KIT-5020) for 30 min at 37˚C via EnVision™+ Dual Link System-HRP (Dako; Agilent Technologies, Inc.). DAB and chromogen solution were used for color reaction enhancement. Hematoxylin was used to counterstain the slides (37˚C for 7 min). A light microscope (BX53, Olympus Corporation) was used to visualize slides and the magnification was x40 (right corner, zoom out) and x200, respectively.

Table II. Summary of patients with NSCLC and non-cancerous control lung tissues in the tissue arrays.

A, NSCLC

Patients characteristics	No. of patients (%)
Age (years)	
<55	118 (45.4)
≥55	142 (54.6)
Sex	
Male	194 (74.6)
Female	66 (25.4)
Clinical stage	
Stage I and II	125 (48.1)
Stage III and IV	125 (51.9)
Lymph node status	
No LNM	111 (42.7)
LNM	149 (57.3)
Histological type	
SCC	129 (49.6)
ADC	131 (50.4)
Differentiation	
Well and moderate	117 (45.0)
Poor	143 (55.0)
Survival state	
Living	160 (61.5)
Mortality	100 (38.5)

B, Non-cancerous lung tissues

Patients characteristics	No. of patients (%)
Age (years)	
<55	25 (48.1)
≥55	27 (51.9)
Sex	
Male	37 (71.2)
Female	15 (28.8)

NSCLC, non-small cell lung cancer; LNM, lymph node metastasis.

NCAPH and Mcl-1 protein expression analysis was performed using semi-quantitative evaluation (28). The staining intensity for NCAPH and Mcl-1 was classified on a scale 0-3 based on the observed color intensity (0=negative, no staining; 1=weak, light brown; 2=moderate, brown and 3=strong, dark brown), and positive rates were evenly divided into five grades [0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%)]. The final staining score for NCAPH and Mcl-1 was determined by the formula: Staining score=positive rates* intensity. The best cut-off score for NCAPH was determined as 4 (high expression was determined when the score was ≥4, while scores <4 were considered negative) based on the survival rate. The optimal cut-off score was determined to be 6

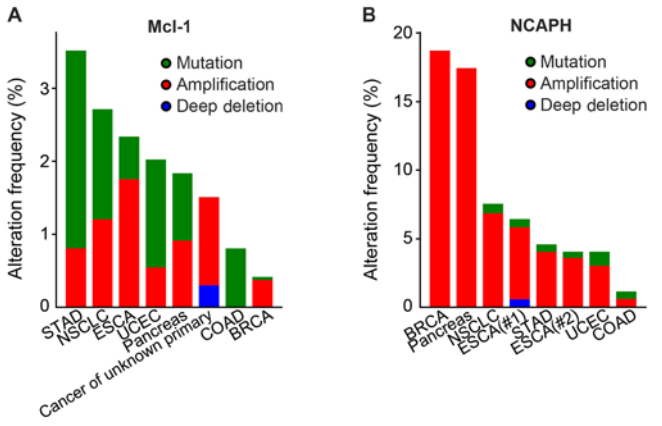

Figure 1. The mutation patterns of (A) Mcl-1 and (B) NCAPH in various types of cancer. NCAPH, Non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; STAD, stomach adenocarcinoma; NSCLC, non-small cell lung cancer; ESCA, esophagogastric carcinoma; UCEC, endometrial cancer; COAD, colorectal cancer; BRCA, breast cancer.

for Mcl-1. The consistency of the two evaluators (Qiuxia Xiong and Songqing Fan) was 95% and differences were solved by discussion between Qiuxia Xiong and Songqing Fan under a two-headed microscope. A light microscope (BX53; Olympus Corporation) was used to visualize slides and the magnification was x40 (right corner, zoom out) and x200, respectively.

Statistical analysis. SPSS version 20.0 was used for statistical analysis (IBM Corp.). The association of NCAPH, Mcl-1, and clinical and pathological characteristics was analyzed via the χ^2 test and verified using The Cancer Genome Atlas (TCGA) database (v21.0; https://portal.gdc.cancer.gov/). The data were generated from the database starBase (v2.0; http://starbase.sysu.edu.cn/panGeneCoExp.php). Overall survival curves were obtained using Kaplan-Meier analysis. The Cox proportional hazard regression model was used for multivariate analysis to determine the potential of positive expression of NCAPH and Mcl-1 as poor prognostic indicators. One-way ANOVA was used for comparisons between groups, and t-test was used for further paired comparisons if differences existed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Protein expression of NCAPH and Mcl-1 in the tissues of LUSC and LUAD. The mutation patterns of NCAPH and Mcl-1 in various cancers were examined (Fig. 1; Table I) (30-37). The result showed that the mutation patterns of NCAPH and Mcl-1 existed in various cancers including stomach adenocarcinoma, non-small cell lung cancer, esophagogastric carcinoma, endometrial cancer, colorectal cancer and breast cancer. Immunohistochemistry was conducted to detect the expression and cellular location of NCAPH and Mcl-1 in LUSC, LUAD and the non-cancerous lung control tissues to investigate the connection of NCAPH and Mcl-1 in malignant tissues. The clinicopathological characteristics are presented in Table II. Expression of NCAPH and Mcl-1 was high in NSCLC tissues and was identified in the cell cytoplasm and nuclear tissues and weak positive expression in the bronchial epithelial cells of non-cancerous normal control lung tissue. In the LUAD and

Table III. Analysis of the association between expression of NCAPH and Mcl-1 proteins and clinicopathological characteristics of NSCLC.

	NCAPH			Mcl-1		
Clinicopathological features	Positive (%)	Negative (%)	P-value	High (%)	Low (%)	P-value
Age (years)			0.302			0.567
<55	76 (64.4)	42 (35.6)		98 (83.1)	20 (16.9)	
≥55	100 (70.4)	42 (29.6)		114 (80.3)	28 (19.7)	
Sex			0.922			0.301
Male	131 (67.5)	64 (32.5)		161 (83.0)	33 (17.0)	
Female	45 (68.2)	21 (31.8)		51 (77.3)	15 (22.7)	
Histological type			0.858			<0.001
ADC	88 (68.2)	41 (31.8)		118 (91.51)	11 (8.5)	
SCC	88 (67.2)	43 (32.8)		94 (71.8)	37 (28.2)	
Clinical stages			0.369			0.768
Stage I and II	88 (70.4)	37 (29.6)		101 (80.8)	24 (19.2)	
Stage III and V	88 (65.2)	47 (34.8)		111 (82.2)	24 (17.8)	
LNM status			0.618			0.421
No LNM	77 (69.4)	34 (39.6)		93 (83.8)	18 (16.2)	
LNM	99 (66.4)	50 (33.4)		119 (79.9)	30 (20.1)	
Pathological grade			0.201			0.247
Well and moderate	84 (71.8)	33 (28.2)		99 (84.6)	18 (15.4)	
Poor	92 (64.3)	51 (35.7)		113 (79.0)	30 (21.0)	
Survival status			0.008[a]			0.034[a]
Alive	118 (73.8)	42 (26.2)		124 (77.5)	36 (22.5)	
Succumbed	58 (58.0)	42 (42.0)		88 (88.0)	12 (12.0)	

[a]P<0.05. Statistical analysis was performed using the χ^2 test. NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; NSCLC, non-small cell lung cancer; ADC, lung adenocarcinoma; SCC, lung squamous carcinoma; LNM, Lymph node metastasis.

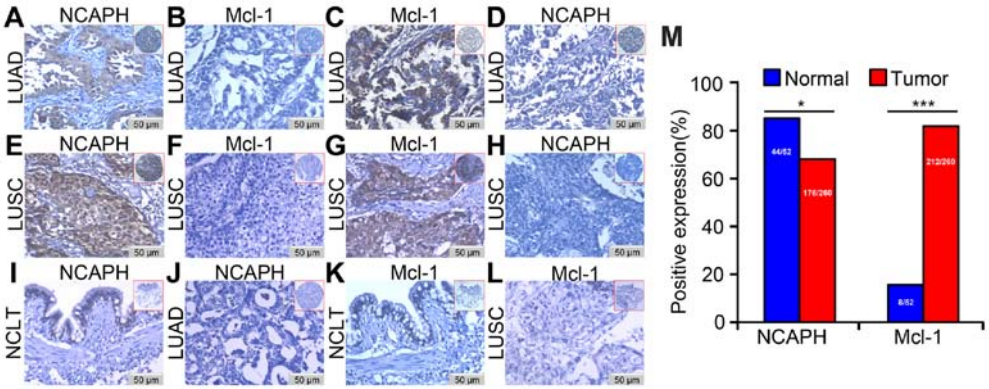

Figure 2. Expression of NCAPH and Mcl-1 proteins in LUAD, LUSC and non-cancerous lung tissues as detected by immunohistochemistry (DAB staining; magnification, x200. The right corner image is a zoomed-out image with magnification, x40). (A) NCAPH and (B) Mcl-1 expression in the matched LUAD tissue. (C) Mcl-1 and (D) NCAPH expression in the matched LUAD tissue. (E) NCAPH and (F) Mcl-1 expression in the matched LUSC tissue. (G) Mcl-1 and (H) NCAPH expression in the matched LUSC tissue. (I) The weak positive NCAPH staining in the NCLT. (J) Negative NCAPH expression in the LUAD tissue. (K) The weak positive Mcl-1 staining in the NCLT. (L) Negative Mcl-1 expression in the LUSC tissue. (M) The percentage of positive expression of NCAPH and Mcl-1 proteins in NSCLC tissues was compared to NCLT. *P<0.05; ***P<0.001; t-test. NCAPH, Non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NCLT, non-cancerous lung tissue.

LUSC tissues, Mcl-1 was negatively expressed in the matched tissue in which NCAPH was positively expressed and vice versa (Fig. 2A-H). A weak positive expression of NCAPH was identified in the control tissues (Fig. 2I). No positive NCAPH staining was observed in the LUAD tissue sections when the matched immunoglobulin G isotype antibody was stained as

Table IV. The pairwise correlation between expression of NCAPH and Mcl-1 proteins in 260 cases of NSCLC.

Protein	NCAPH	Mcl-1
NCAPH		
Spearman's correlation coincident	1	-0.185
Significance (2-tailed)	-	0.003[a]
Mcl-1		
Spearman's correlation coincident	-0.185	1
Significance (2-tailed)	0.003[a]	-

[a]P<0.05. NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; NSCLC, non-small cell lung cancer; (-), not available.

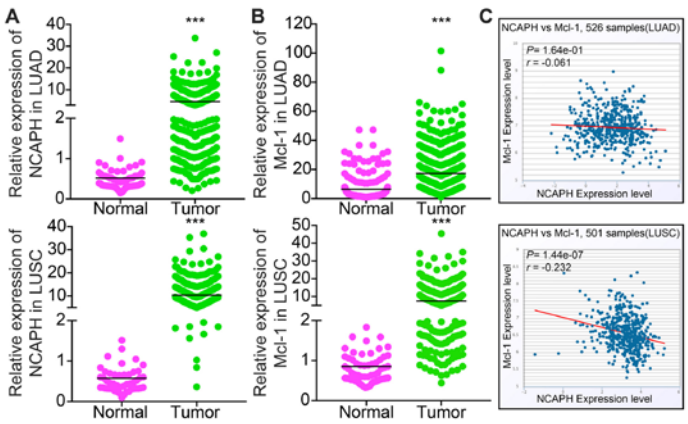

Figure 3. Elevated expression of NCAPH and Mcl-1 in NSCLC. Significant differential expression of (A) NCAPH and (B) Mcl-1 between tumor and normal tissues in LUAD and LUSC from The Cancer Genome Atlas data. (C) NCAPH association with Mcl-1 in LUAD and LUSC tissues. Data were generated from http://starbase.sysu.edu.cn/panGeneCoExp.php. ***P<0.001; t-test. NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

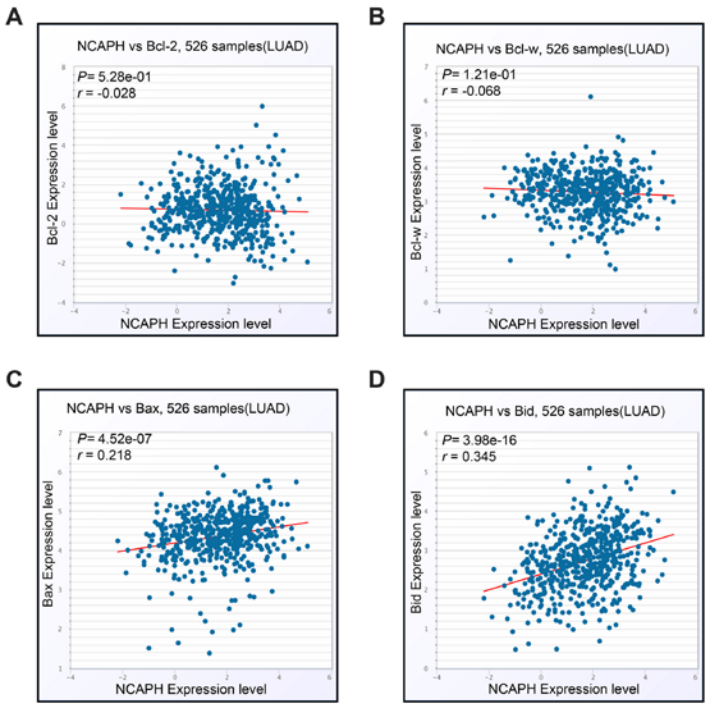

Figure 4. The associations between NCAPH and other Bcl-2 members. NCAPH negatively associates with (A) Bcl-2 and (B) Bcl-w in LUAD. NCAPH positively associates with (C) Bax and (D) Bid in LUAD, and P-values are indicated in each figure. NCAPH, non-SMC condensin I complex subunit H; LUAD, lung adenocarcinoma; Bid, BH3 interacting-domain death agonist.

Table V. Summary of multivariate analysis of Cox proportional hazard regression for overall survival in 260 cases of patients with NSCLC.

Variable	Wald	Sig.	Exp (B)	95.0% CI for Exp (B) Lower	Upper
Age	0.001	0.976	1.006	0.667	1.517
Sex	2.442	0.118	0.673	0.409	1.106
Histological types	3.285	0.070	1.516	0.967	2.376
Pathological grades	5.746	0.017[a]	1.689	1.100	2.593
LNM	3.819	0.051	1.595	0.999	2.547
Clinical stages	8.982	0.003[a]	2.055	1.283	3.291
Treatment strategy	0.087	0.768	0.918	0.519	1.624
NCAPH	8.539	0.003[a]	0.544	0.361	0.818
Mcl-1	4.324	0.038[a]	1.955	1.039	3.678

Multivariate analysis of Cox regression, [a]P<0.05. NSCLC, non-small cell lung cancer; LNM, lymph node metastasis; CI, confidence interval; NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1.

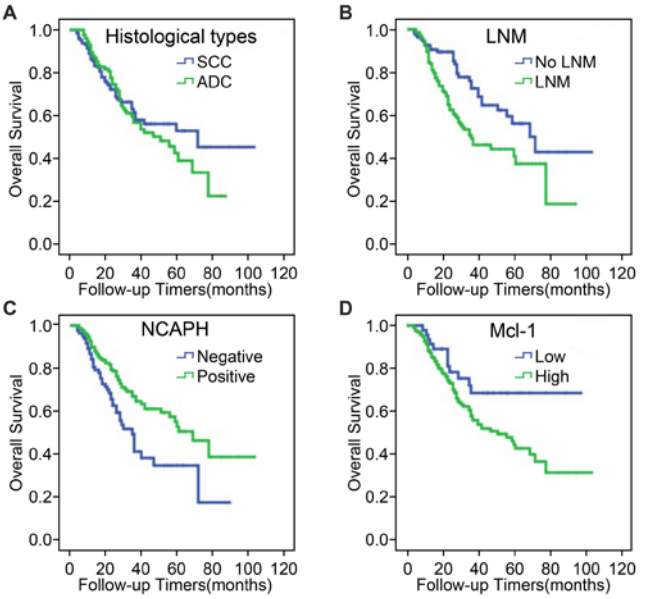

Figure 5. Kaplan-Meier curves were used to compare the overall survival curves of patients with NSCLC according to clinicopathological characteristics and expression of NCAPH and Mcl-1 proteins. (A) Kaplan-Meier analysis of the patients with LUAD (green) and LUSC (blue) from tissue microarrays used in the current study, P=0.554. (B) Kaplan-Meier analysis of the patients with lymph node metastasis (green) and no lymph node metastasis (blue) from tissue microarrays used in the current study, P=0.002. (C) Kaplan-Meier analysis of the association between NCAPH expression and the overall survival (blue, negative expression of NCAPH; green, positive expression of NCAPH), P=0.003. (D) Kaplan-Meier analysis of the association between Mcl-1 expression and the overall survival (blue, low expression of Mcl-1; green, high expression of Mcl-1), P=0.032. NSCLC, non-small cell lung cancer; NCAPH, non-SMC condensin I complex subunit H; Mcl-1, myeloid cell leukemia sequence 1; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; LNM, lymph node metastasis; SCC, lung squamous carcinoma; ADC, lung adenocarcinoma.

a negative control (Fig. 2J). Weak positive expression of Mcl-1 was revealed in the bronchial epithelial cells of non-cancerous control normal lung tissue (Fig. 2K). The negative control demonstrated no expression of Mcl-1 in LUSC (Fig. 2L).

The expression of NCAPH and Mcl-1 in the non-cancerous control lung and NSCLC tissues was quantified (Fig. 2M); the positive percentage of NCAPH in the non-cancerous lung tissues (84.61%: 44/52) was higher than that in NSCLC (67.69%; 176/260; P=0.015). The positive percentage of Mcl-1 in the non-cancerous lung tissues (15.38%; 8/52) was lower compared with that in NSCLC (81.54%; 212/260; P<0.001). The aforementioned results indicated that the positive expression percentage of NCAPH was lower in tissues harvested from patients with patients while the positive percentages of Mcl-1 were significantly higher compared with control lung tissue sections.

Association between NCAPH and Mcl-1 and the clinical characteristics in NSCLC. The univariate χ^2 test was used to examine the influence of the altered expression of NCAPH and Mcl-1 in NSCLC tissues on clinical outcomes. The clinicopathological features investigated included age, clinical stage, sex, lymph node (LNM) status, pathological grade and survival state. The high percentage of Mcl-1 expression was significantly associated with histological type (P<0.0001; Table III). The positive percentages of NCAPH were significantly increased in the living group (73.8 vs. 58.0%; P=0.008), while the high percentage of Mcl-1 expression was significantly higher in the mortality group (88.0 vs. 77.5%; P=0.034). However, no association was observed between the expression of NCAPH/Mcl-1 and sex, age, LNM stage or pathological grade.

The pairwise association between NCAPH and Mcl-1 proteins in NSCLC. Pairwise association (Table IV) demonstrated that positive expression of NCAPH protein was associated with negative expression of Mcl-1 protein (r=-0.185, P=0.003), while the positive expression of Mcl-1 protein was associated with negative expression of NCAPH protein (r=-0.185, P=0.003). Therefore, the overexpression of NCAPH protein was negatively associated with the expression of Mcl-1. To evaluate the expression profiles of NCAPH and Mcl-1 in LUAD and LUSC, The Cancer Genome Atlas (TCGA) database was analyzed. The results showed that NCAPH is highly expressed in LUAD and LUSC (Fig. 3A), and Mcl-1 is highly expressed in LUAD and LUSC (Fig. 3B). In addition, NCAPH expression is negative associated with Mcl-1, as validated in LUAD and LUSC by utilizing the TCGA dataset (Fig. 3C) (38). In addition, NCAPH was revealed to be negatively associated with pro-survival members including Bcl-2 and Bcl-w, while being positively associated with pro-apoptotic members Bax and Bid (Fig. 4A-D).

Impact of NCAPH and Mcl-1 expression levels on the prognosis in NSCLC. To serve as a control, no significant difference was detected in the overall survival (OS) between patients with LUAD and LUSC from tissue microarrays used in the current study (Fig. 5A; P=0.554). The results of Kaplan-Meier curves indicated that the overall survival rates for patients with lymph node metastasis were lower than for metastasis-free patients

(P=0.002; Fig. 5B). Meanwhile, higher NCAPH expression in NSCLC was associated with higher OS rates (P=0.003; Fig. 5C), while patients with high Mcl-1 expression in NSCLC exhibited significantly worse OS rates (P=0.032; Fig. 5D).

Multivariate Cox proportional hazard regression analysis was performed on 260 NSCLC cases. Pathological grades (P=0.017) and clinical stages (P=0.003) were revealed to serve as prognostic factors (Table V). Additionally, the expression of NCAPH (P=0.003) and Mcl-1 (P=0.038) could be considered as distinct prognostic factors in patients with NSCLC. No clinical effect based on age, sex, histological types, LNM, or treatment strategy was detected.

Discussion

NCAPH is one of the members of the barr gene family and is located on chromosome 2q11.2 (39). A previous study has indicated that NCAPH is one of the essential factors for maintaining cell survival and is indispensable in mitotic chromosome cohesion and separation (40). Mcl-1 is homologous to Bcl-2 and possesses an anti-apoptotic effect in regulating cell survival (41). Compared with healthy lungs, the overexpression of Mcl-1 in NSCLC lines is associated with poor patient prognosis. Mcl-1 belongs to the pro-apoptotic Bcl-2 family, maintains its inactive monomeric state and antagonizes the signaling of cellular apoptosis, particularly in Mcl-1-overexpressing NSCLC cell lines (23). In addition, the depletion of Mcl-1 results in increased sensitivity to radiotherapy and chemotherapy in NSCLC cells (42). Huang *et al* (43) revealed that Mcl-1 promotes the migration of lung cancer cells through a mechanism involving Ca^{2+}-dependent reactive oxygen species production. Therefore, Mcl-1 has been demonstrated to serve an important role in NSCLC cell survival by limiting apoptotic signaling.

In the present study, the protein expression of NCAPH and Mcl-1 were examined by IHC in tissues of 260 cases of NSCLC. The data demonstrated that NCAPH and Mcl-1 were highly expressed in NSCLC cancerous tissues; with positive expression in the cytoplasm and nucleus and weak positive expression in the bronchial epithelial cells of non-cancerous normal control lung tissue. The expression of NCAPH and Mcl-1 was negatively associated in the matched tissues of LUAD and LUSC. Pairwise association also demonstrated that NCAPH and Mcl-1 overexpression were inversely associated.

Precise biomarkers are essential for providing a reference for NSCLC prognosis in the tumor-node-metastasis staging system; one of the most effective prognostic methods for operable NSCLC (42). Therefore, it is important to identify novel biomarkers that can help to determine the risks of tumor occurrence and progression. According to the association analysis data between NCAPH and Mcl-1, the high expression percentage of Mcl-1 was positively associated with histological type in NSCLC. In addition, the positive percentage expression of NCAPH was significantly elevated in the living group, while the percentage expression of Mcl-1 was increased in the mortality group. Positive NCAPH expression was associated with higher OS rates, whereas, high Mcl-1 expression was inversely associated with OS rates. These results indicated that the expression of NCAPH and Mcl-1 may be associated with the development and progression of NSCLC and can be considered to be independent prognostic factors. Multiple regulatory mechanisms including ubiquitination and subsequent proteasomal degradation have been shown to control Mcl-1 functions by regulating its expression in response to different stimuli (44). Recent studies validate the non-apoptotic functions of Mcl-1 in autophagy, mitochondrial homeostasis and protein kinase cascade signaling (45). Mcl-1 physically interacts with a number of cell cycle regulators in the nucleus (including PCNA and Chk1), thus regulating the checkpoint response (46,47). This leads to the hypothesis that NCAPH could also form a complex with, and negatively regulate, Mcl-1 expression during NSCLC progression. This hypothesis should be investigated in future studies due to the lack of current proteasome data from the NCAPH complex. In conclusion, the expression of NCAPH protein was associated with the expression of Mcl-1 in patients with NSCLC, and the positive expression of NCAPH may serve as one of the independent prognostic factors in surgically resected patients with this disease.

Acknowledgements

The authors would like to thank Dr Nigel W. Fraser, Dept of Microbiology, Pereleman School of Medicine, University of Pennsylvania, USA, for his instructive comments on the writing of the manuscript.

Funding

The present study was supported by Yunnan Provincial Department of science and Technology-Kunming Medical University applied basic research joint project in 2019 (grant no. 2019FE001 (-042)) and Scientific research projects of research institutions in medical and health units of Yunnan Province in 2018 (grant no. 2018NS0111).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QX, YD, SF, LD and XC conceived and designed the experiments. YD, QX, SF, BL, XJ, CX, QT, HZ and JW performed the experiments. QX, SF, CX, CC and JW analyzed the data. YD, QX, SF, XC, JW, HZ, YD and QT contributed reagents, materials or analytical tools. YD, QX, SF, LD, QT and CX wrote the manuscript. QX, SF, XC and BL accessed the full-text articles. XJ generated the revised Fig. 4. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Scientific and Research Ethics Committee of the Second Xiangya Hospital (approval no. S039/2011). Patients were fully informed about specimen usage and data retrieval prior to the acquisition of specimens. Written informed consent was obtained from each patient. Informed consent was obtained from a parent and/or legal guardian for subjects <18 years old.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Torre LA, Siegel RL, Ward EM and Jemal A: Global cancer incidence and mortality rates and trends-an update. Cancer Epidemiol Biomarkers Prev 25: 16-27, 2016.
2. Chen M, Liu X, Du J, Wang XJ and Xia L: Differentiated regulation of immune-response related genes between LUAD and LUSC subtypes of lung cancers. Oncotarget 8: 133-144, 2017.
3. Yoshioka H, Shimokawa M, Seto T, Morita S, Yatabe Y, Okamoto I, Tsurutani J, Satouchi M, Hirashima T, Atagi S, *et al*: Final overall survival results of WJTOG3405, a randomized phase III trial comparing gefitinib versus cisplatin with docetaxel as the first-line treatment for patients with stage IIIB/IV or postoperative recurrent EGFR mutation-positive non-small-cell lung cancer. Ann Oncol 30: 1978-1984, 2019.
4. Bobbili P, Ryan K, Duh MS, Dua A, Fernandes AW, Pavilack M and Gomez JE: Treatment patterns and overall survival among patients with unresectable, stage III non-small-cell lung cancer. Future Oncol 15: 3381-3393, 2019.
5. León-Atance P, Moreno-Mata N, González-Aragoneses F, Cañizares-Carretero MA, García-Jiménez MD, Genovés-Crespo M, Honguero-Martínez AF, Rombolá CA, Simón-Adiego CM and Peñalver-Pascual R: Multicenter analysis of survival and prognostic factors in pathologic stage I non-small-cell lung cancer according to the new 2009 TNM Classification. Arch Bronconeumol 47: 441-446, 2011 (In English, Spanish).
6. Criss SD, Mooradian MJ, Watson TR, Gainor JF, Reynolds KL and Kong CY: Cost-effectiveness of atezolizumab combination therapy for first-line treatment of metastatic nonsquamous non-small cell lung cancer in the United States. JAMA Netw Open 2: e1911952, 2019.
7. Rosser CJ and Goodison S: CD24, a promising biomarker in NSCLC. Biomark Med 4: 495, 2010.
8. Zhan SJ, Liu B and Linghu H: Identifying genes as potential prognostic indicators in patients with serous ovarian cancer resistant to carboplatin using integrated bioinformatics analysis. Oncol Rep 39: 2653-2663, 2018.
9. Sun C, Huang S, Wang H, Xie R, Zhang L, Zhou Q, He X and Ju W: Non-SMC condensin I complex subunit H enhances proliferation, migration, and invasion of hepatocellular carcinoma. Mol Carcinog 58: 2266-2275, 2019.
10. Hirano T: Condensins: Universal organizers of chromosomes with diverse functions. Genes Dev 26: 1659-1678, 2012.
11. Wood AJ, Severson AF and Meyer BJ: Condensin and cohesin complexity: The expanding repertoire of functions. Nat Rev Genet 11: 391-404, 2010.
12. Kimura K, Cuvier O and Hirano T: Chromosome condensation by a human condensin complex in xenopus egg extracts. J Biol Chem 276: 5417-5420, 2001.
13. Martin CA, Murray JE, Carroll P, Leitch A, Mackenzie KJ, Halachev M, Fetit AE, Keith C, Bicknell LS, Fluteau A, *et al*: Mutations in genes encoding condensin complex proteins cause microcephaly through decatenation failure at mitosis. Genes Dev 30: 2158-2172, 2016.
14. Yin L, Jiang LP, Shen QS, Xiong QX, Zhuo X, Zhang LL, Yu HJ, Guo X, Luo Y, Dong J, *et al*: NCAPH plays important roles in human colon cancer. Cell Death Dis 8: e2680, 2017.
15. Ryu B, Kim DS, Deluca AM and Alani RM: Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. PLoS One 2: e594, 2007.
16. Xu L, Jiang Y, Zheng J, Xie G, Li J, Shi L and Fan S: Aberrant expression of β-catenin and e-cadherin is correlated with poor prognosis of nasopharyngeal cancer. Hum Pathol 44: 1357-1364, 2013.
17. Granville CA and Dennis PA: An overview of lung cancer genomics and proteomics. Am J Respir Cell Mol Biol 32: 169-176, 2005.
18. Kozopas KM, Yang T, Buchan HL, Zhou P and Craig RW: MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc Natl Acad Sci USA 90: 3516-3520, 1993.
19. Han Y, Wu N, Jiang M, Chu Y, Wang Z, Liu H, Cao J, Liu H, Xu B and Xie X: Long non-coding RNA MYOSLID functions as a competing endogenous RNA to regulate MCL-1 expression by sponging miR-29c-3p in gastric cancer. Cell Prolif 52: e12678, 2019.
20. Kour S, Rana S, Contreras JI, King HM, Robb CM, Sonawane YA, Bendjennat M, Crawford AJ, Barger CJ, Kizhake S, *et al*: CDK5 inhibitor downregulates Mcl-1 and sensitizes pancreatic cancer cell lines to navitoclax. Mol Pharmacol 96: 419-429, 2019.
21. Meister MT, Boedicker C, Linder B, Kogel D, Klingebiel T and Fulda S: Concomitant targeting of Hedgehog signaling and MCL-1 synergistically induces cell death in Hedgehog-driven cancer cells. Cancer Lett 465: 1-11, 2019.
22. Akgul C: Mcl-1 is a potential therapeutic target in multiple types of cancer. Cell Mol Life Sci 66: 1326-1336, 2009.
23. Zhang H, Guttikonda S, Roberts L, Uziel T, Semizarov D, Elmore SW, Leverson JD and Lam LT: Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines. Oncogene 30: 1963-1968, 2011.
24. Whitsett TG, Mathews IT, Cardone MH, Lena RJ, Pierceall WE, Bittner M, Sima C, LoBello J, Weiss GJ and Tran NL: Mcl-1 mediates TWEAK/Fn14-induced non-small cell lung cancer survival and therapeutic response. Mol Cancer Res 12: 550-559, 2014.
25. Yang S, Kim CY, Hwang S, Kim E, Kim H, Shim H and Lee I: COEXPEDIA: Exploring biomedical hypotheses via co-expressions associated with medical subject headings (MeSH). Nucleic Acids Res 45(D1): D389-D396, 2017.
26. Travis WD, Brambilla E, Burke AP, Marx A and Nicholson AG (eds): WHO Classification of tumours of the lung, pleura, thymus and heart. In: WHO Classification of Tumours. Vol 7. 4th edition. IARC, Lyon, 2015.
27. Boffa DJ and Greene FL: Reacting to changes in staging designations in the 7th edition of the AJCC staging manual. Ann Surg Oncol 18: 1-3, 2011.
28. Wen Q, Wang W, Luo J, Chu S, Chen L, Xu L, Zang H, Alnemah MM, Ma J and Fan S: CGP57380 enhances efficacy of RAD001 in non-small cell lung cancer through abrogating mTOR inhibition-induced phosphorylation of eIF4E and activating mitochondrial apoptotic pathway. Oncotarget 7: 27787-27801, 2016.
29. Wen Q, Wang W, Chu S, Luo J, Chen L, Xie G, Xu L, Li M and Fan S: Flot-2 expression correlates with EGFR levels and poor prognosis in surgically resected non-small cell lung cancer. PLoS One 10: e0132190, 2015.
30. Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, Schumacher SE, Wang C, Hu H, Liu J, *et al*: Genomic and functional approaches to understanding cancer aneuploidy. Cancer Cell 33: 676-689.e673, 2018.
31. Jordan EJ, Kim HR, Arcila ME, Barron D, Chakravarty D, Gao J, Chang MT, Ni A, Kundra R, Jonsson P, *et al*: Prospective comprehensive molecular characterization of lung adenocarcinomas for efficient patient matching to approved and emerging therapies. Cancer Discov 7: 596-609, 2017.
32. Janjigian YY, Sanchez-Vega F, Jonsson P, Chatila WK, Hechtman JF, Ku GY, Riches JC, Tuvy Y, Kundra R, Bouvier N, *et al*: Genetic predictors of response to systemic therapy in esophagogastric cancer. Cancer Discov 8: 49-58, 2018.
33. Soumerai TE, Donoghue MTA, Bandlamudi C, Srinivasan P, Chang MT, Zamarin D, Cadoo KA, Grisham RN, O'Cearbhaill RE, Tew WP, *et al*: Clinical utility of prospective molecular characterization in advanced endometrial cancer. Clin Cancer Res 24: 5939-5947, 2018.
34. Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, *et al*: Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 6: 6744, 2015.
35. Ghandi M, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER III, Barretina J, Gelfand ET, Bielski CM, Li H, *et al*: Next-generation characterization of the cancer cell line encyclopedia. Nature 569: 503-508, 2019.
36. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, Jayakumaran G, Middha S, Zehir A, Donoghue MTA, *et al*: Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer Cell 33: 125-136.e3, 2018.

37. Pereira B, Chin SF, Rueda OM, Vollan HK, Provenzano E, Bardwell HA, Pugh M, Jones L, Russell R, Sammut SJ, *et al*: The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 7: 11479, 2016.
38. Li JH, Liu S, Zhou H, Qu LH and Yang JH: StarBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 42 (Database Issue): D92-D97, 2014.
39. Neuwald AF and Hirano T: HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. Genome Res 10: 1445-1452, 2000.
40. Lawrimore J and Bloom K: The regulation of chromosome segregation via centromere loops. Crit Rev Biochem Mol Biol 54: 352-370, 2019.
41. Senichkin VV, Streletskaia AY, Zhivotovsky B and Kopeina GS: Molecular comprehension of Mcl-1: From gene structure to cancer therapy. Trends Cell Biol 29: 549-562, 2019.
42. Song L, Coppola D, Livingston S, Cress D and Haura EB: Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells. Cancer Biol Ther 4: 267-276, 2005.
43. Huang H, Shah K, Bradbury NA, Li C and White C: Mcl-1 promotes lung cancer cell migration by directly interacting with VDAC to increase mitochondrial Ca2+ uptake and reactive oxygen species generation. Cell Death Dis 5: e1482, 2014.
44. Mojsa B, Lassot I and Desagher S: Mcl-1 ubiquitination: Unique regulation of an essential survival protein. Cells 3: 418-437, 2014.
45. Young AI, Timpson P, Gallego-Ortega D, Ormandy CJ and Oakes SR: Myeloid cell leukemia 1 (MCL-1), an unexpected modulator of protein kinase signaling during invasion. Cell Adh Migr 12: 513-523, 2018.
46. Fujise K, Zhang D, Liu J and Yeh ET: Regulation of apoptosis and cell cycle progression by MCL1: Differential role of proliferating cell nuclear antigen. J Biol Chem 275: 39458-39465, 2000.
47. Jamil S, Mojtabavi S, Hojabrpour P, Cheah S and Duronio V: An essential role for MCL-1 in ATR-mediated CHK1 phosphorylation. Mol Biol Cell 19: 3212-3220, 2008.