



# Non-SMC condensin I complex subunit H (NCAPH), a regulator of cell cycle, predicts poor prognosis in lung adenocarcinoma patients: a study mainly based on TCGA and GEO database

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**Background:** Lung adenocarcinoma (LUAD) is the main sub-type of lung cancer, which is a major disease of human death. However, the role of non-SMC condensin I complex subunit H (NCAPH) in LUAD and its possible upstream regulation microRNAs (miRNAs) remains unclearly.

**Methods:** In this study, we analyzed the NCAPH mRNA and protein expression in normal and cancer tissues mainly based on Human Protein Atlas (HPA) database, The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. With the help of the Kaplan Meier plotter, we explored the prognosis role in LUAD. Furtherly, the co-expressed genes of NCAPH in LUAD were obtained by using cBioPortal, GEPIA and UALCAN database. Then, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of co-expression genes of NCAPH was conducted by DAVID, while the protein-protein interaction (PPI) network was constructed with STRING and hub genes were identified and visualized by Cytoscape software. We also investigated the miRNAs and chemicals that may downregulated the NCAPH expression.

**Results:** The results showed that NCAPH expression level was elevated in LUAD tissue compared with normal lung tissue and predicted poor prognosis. GO and KEGG pathway enriched analysis of co-expressed genes suggested that NCAPH may play an important role in cell cycle in LUAD. Nine top hub co-expressed genes were all negatively related to the LUAD prognosis. Lastly, 8 miRNAs and 5 chemicals were identified to have the potential to down-regulate the NCAPH expression.

**Conclusions:** Our study indicated that NCAPH expression in LUAD is a poor prognostic indicator, which may be the potential therapeutic target in the future.

**Keywords:** lung adenocarcinoma (LUAD); non-SMC condensin I complex subunit H (NCAPH); miRNA; prognosis

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## Introduction

Lung cancer, the leading cause of cancer-related mortality, is a major disease that threatens human health (1-4). As far as

the progression in surgery, radiotherapy and chemotherapy, the 5-year survival rate of lung cancer is only 15% (5). However, the adoption of immunotherapies and targeted

therapies have increased the 5-year survival rate obviously (range from 15% to 50%) (6,7). Lung adenocarcinoma (LUAD) is the main sub-type of lung cancer, and in recent years the incidence of LUAD has increased distinctly. So, it's obliged to find new therapeutic targets for LUAD.

Condensin, a multiprotein complex, serves as a regulator of chromosome-wide gene. During mitosis and meiosis, It plays an important role in the process of chromosome assembly and segregation, regulating the cell cycle (8). There are two kinds of condensin complex: condensin I and condensin II. Condensing I complex is composed of structural maintenance of chromosomes (SMC) proteins and three non-SMC subunits, including non-SMC condensin I complex subunit H (NCAPH), subunit I (NCAPG) and subunit D2 (NCAPD2) (9). NCAPH is up-regulated in many kinds of tumor tissue, such as prostate cancer, pancreatic cancer and hepatocellular carcinoma, and it often predicts poor prognosis (10-12), regulating the cell cycle, migration and invasion. These studies proved that NCAPH could be an important therapeutic target for cancer. But in colon cancer, NCAPH is positively correlated with prognosis (13). Till now, the exact role of NCAPH in LUAD and its possible upstream regulation mechanism remain unclearly.

In order to answer this question, The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) database were analyzed with online tools. We demonstrate that NCAPH is up-regulated in LUAD compared with adjacent normal tissues. What's more, the survival analysis revealed that LUAD patients with high level of NCAPH had worse survival time. Potential mechanism and its upstream microRNAs (miRNAs) were also analyzed by online tools, such as cBioPortal, GEPIA, UALCAN and TargetScan. In conclusion, our study offers a new potential therapeutic target for the treatment of LUAD. We present the following article in accordance with the TRIPOD reporting checklist (available at <http://dx.doi.org/10.21037/tcr-20-2217>).

## Methods

### *Analysis of TCGA and GEO database*

TCGA database (<https://www.cancer.gov/tcga>), a landmark cancer genomics program, was created by the National Cancer Institute and the National Human Genome Research Institute in 2006. It includes genomic, epigenomic, transcriptomic, and proteomic data of 33

cancer types, as well as the matched normal samples. In order to view the expression profile of NCAPH in pan-cancer, we use a web-based database-GEPIA (<http://gepia.cancer-pku.cn>). This database could analyze the RNA sequencing expression data from the TCGA and the GTEx projects (14). For LUAD patients, the relationship between clinicopathological parameters and NCAPH expression was explored by UALCAN (<http://ualcan.path.uab.edu/index.html>) (15), which is an interactive and user-friendly web resource mainly based on TCGA data. The enrolled clinicopathological features included gender, age, tumor stage, lymph node metastasis status and smoking history.

GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) was furtherly mined for demonstrate the deferent expression level of NCAPH mRNA between the LUAD tissue and normal lung tissue. GEO database is a good helper for providing users with array- and sequence-based data. In this study, the RNA-seq data of GSE19188 (16), GSE7670 (17) and GSE10072 (18) were extracted and analyzed by GEO2R online tool. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

### *Human Protein Atlas (HPA) database analysis*

Expression level of NCAPH mRNA and protein in human normal organs were retrieved by HPA database (<https://www.proteinatlas.org>) (19). This database contains immunohistochemistry (IHC) staining pictures of 20 LUAD tissues and 6 normal lung tissues. According to the IHC staining intensity, the picture was scored from 0 to 3 (0, negative; 1, weak staining; 2, moderate staining; 3, strong staining). Based on the percentage of stained cells, the picture was scored from 0 to 3 (0, none; 1, <25%; 2, 25-75%; 3, >75%). The IHC score for every picture was calculated by multiplying the staining intensity score with the staining extent score, so the IHC score ranges from 0 to 12 for each sample.

### *The Kaplan Meier plotter database analysis*

The Kaplan Meier plotter (<https://kmplot.com/analysis/>) includes gene chip and RNA-seq data of 20 different cancer types. The system data mainly comes from GEO and TCGA database, which can make a meta-analysis based discovery and validation of survival biomarkers (20). By using the Kaplan Meier plotter database, we examined the relationship between NCAPH mRNA expression and the overall survival

(OS) time of 720 LUAD patients. All the patients were divided into two groups (high vs. low) by median expression value of NCAPH mRNA. Then the web-based tool can calculate the log-rank P value, hazard ratio (HR), and 95% confidence interval (CI) directly. OS time, FP (first progression time), and PPS (post progression survival time) were used to estimate the prognosis of LUAD.

### ***GO and KEGG pathway analysis of co-expression genes of NCAPH***

Firstly, we explored the co-expressed genes of NCAPH in LUAD patients using cBioPortal database (<http://www.cbioportal.org/>). With the help of cBioPortal database, we can download the co-expressed genes of NCAPH in LUAD patients based on TCGA data (21). In order to obtain the accurate co-expressed genes, we used two other databases: GEPIA and UALCAN. Then we intersected the results obtained from these three databases through a website tool-VENNY (<https://bioinfogp.cnb.csic.es/tools/venny/>).

Secondly, for the aim to understand the potential biological process and pathway, the terminal results of co-expressed genes were furtherly processed with gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov>) was accepted for these analyses (22). We visualized the GO and KEGG analysis results using R/Bio conductor (version 3.26.5).

### ***PPI network construction and hub-gene analysis***

STRING (version 11.0) is a web-based tool (<https://string-db.org>) for exploring the known and predicted protein-protein interactions (PPI) (23). We inputted the co-expressed genes of NCAPH in LUAD patients into the STRING database, then it could analyze the PPI network automatically. In order to get the hub-genes in the PPI network, the cytoHubba app of Cytoscape software (version 3.6.1) was utilized. The top nine genes ranked by degree were calculated and visualized (24).

### ***Prediction of potential miRNAs that regulate NCAPH***

TargetScan (version 7.2, [http://www.targetscan.org/vert\\_72/](http://www.targetscan.org/vert_72/)) predicts potential miRNAs for mRNA by searching for the seed region of miRNA that matches to

human 3' UTRs (25). OncomiR database (<http://www.oncomir.org/>) is an open-source platform for pan-cancer miRNA dysregulation in cancer (26). MiRNAs and its target genes are negatively correlated. So, we searched OncomiR database for down-regulated miRNAs in LUAD tissues. To further narrow down the range of target genes, miRNAs positively related with survival time were also analyzed using OncomiR database. Then, we got 9 common miRNAs of the three data sets. ENCORI (<http://starbase.sysu.edu.cn/>) was an online tool for analyzing TCGA RNA-Seq data of many kinds of cancers including LUAD (27). Then, we used ENCORI database to assess the expression correlation of NCAPH mRNA and these 9 miRNAs.

### ***The analysis of potential chemicals that down-regulate NCAPH***

CTD (<http://ctdbase.org/>) is a publicly available database that can be used to analyze the chemical-gene interactions (28). We searched chemicals that could down-regulate NCAPH mRNA or protein. Chemicals with at least 2 references were treated as potential drugs. The structure of potential chemicals was furtherly downloaded by using PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).

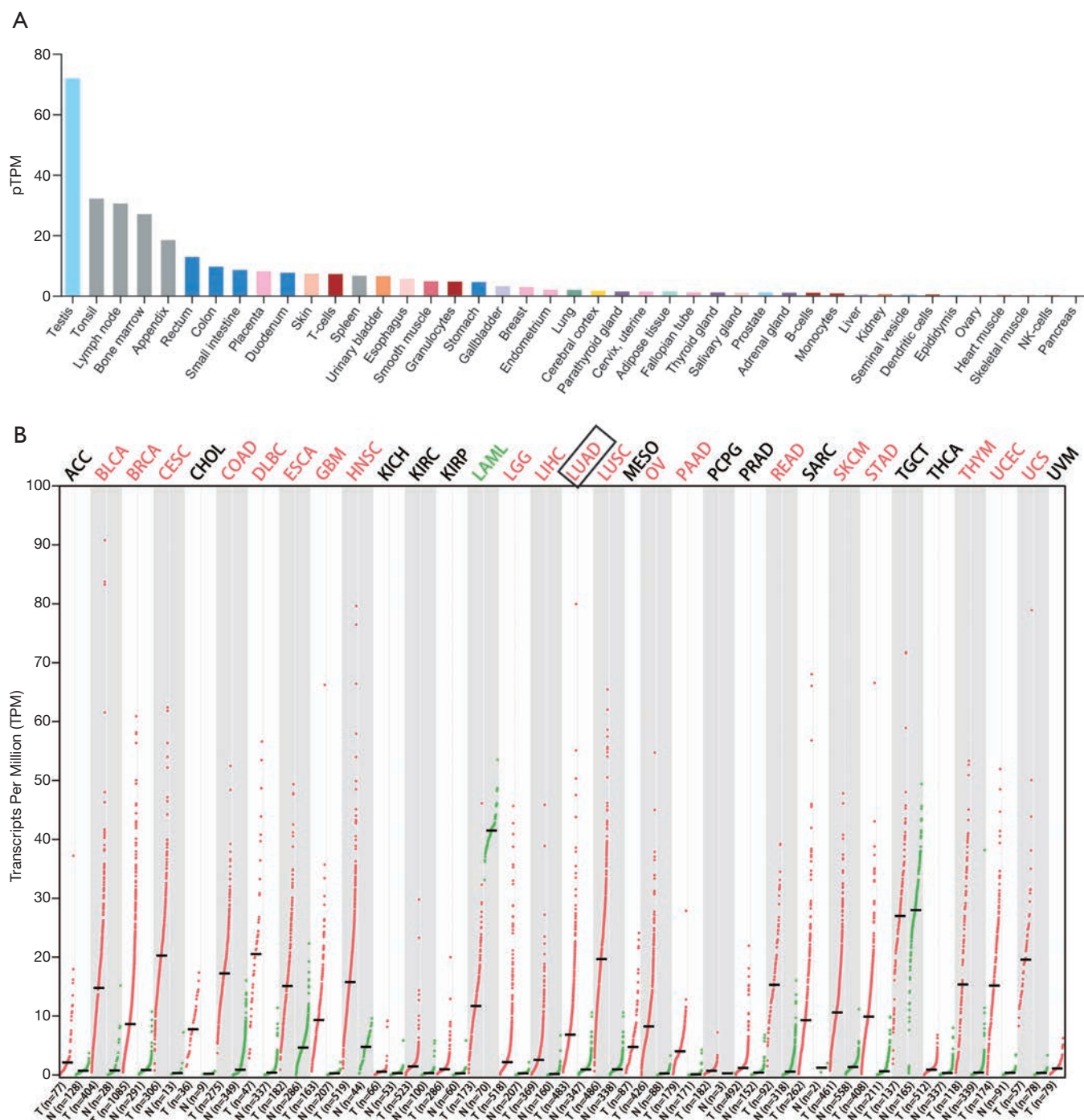
### ***Statistical analysis***

Statistics analysis were carried out using GraphPad Prism 7, and the results are presented as the mean  $\pm$  SD. Differences between two groups was evaluated by Student's *t*-test (unpaired, two-tailed). One-way ANOVA was used for the data subject to normal distribution.  $P < 0.05$  was considered significant.

## **Results**

### ***Expression levels of NCAPH in human normal and cancer tissues***

In order to clarify the expression level of NCAPH mRNA in normal tissues, the HPA database was used. The results proved that the level of NCAPH mRNA was very low in many normal tissues, such as lung, cerebral cortex, adipose tissue, cervix, and uterine, etc. (*Figure 1A*). However, in LUAD as well as many other types of cancers, the NCAPH mRNA expression level increased significantly in comparison with normal tissues (*Figure 1B*).



**Figure 1** Expression level of NCAPH mRNA in normal and cancer tissues. (A) Expression profile of NCAPH mRNA in normal tissues based on Human Protein Atlas (HPA) database. (B) Expression profile of NCAPH mRNA in cancer tissues based on GEPIA database. Black box pointed NCAPH mRNA expression level in lung adenocarcinoma (LUAD).



### ***Elevated NCAPH mRNA in LUAD based on TCGA and GEO***

UALCAN, the online analysis tool of TCGA, showed that NCAPH mRNA expression level was higher in LUAD (Figure 2A). Then we investigated the relationship between the NCAPH mRNA in LUAD and the clinicopathological parameters. LUAD patients from stage I to stage IV got a higher level of NCAPH mRNA compared with normal lung tissues (Figure 2B). The results showed that patients with all kinds of subtype such as nodal metastasis status (Figure 2C), gender (Figure 2D), age (Figure 2E), and smoking habits (Figure 2F) got higher NCAPH mRNA levels compared with normal lung tissues. We also explored the expression of NCAPH mRNA in GEO database. GSE19188, GSE7670 and GSE10072 were enrolled in our study. NCAPH mRNA levels were relatively higher in LUAD patients in the three datasets (Figure 2G,H,I).

### ***Increased expression of NCAPH protein in LUAD***

Proteins of cells are the fundamental of biological function. In order to study the expression of NCAPH protein, we check the HPA database. There are two kinds of antibody for NCAPH: HPA002647 and HPA003008. Each antibody staining group concluded 3 IHC images of normal lung tissue and 10 IHC images of LUAD tissue. As shown in Figure 3, the IHC images of LUAD got a higher IHC score compared with normal tissues. This result demonstrated that the expression level of NCAPH protein was increased in LUAD tissues in comparison with normal lung tissues.

### ***The prognostic role of NCAPH in LUAD patients***

The prognostic role of NCAPH In LUAD patients was mined by using the Kaplan Meier plotter database. The probe name of NCAPH was 212949\_at. As shown in Figure 4, increased level of NCAPH indicated a significantly worse prognosis in the aspects of OS time (Figure 4A) and FP time (Figure 4B). But, no significant difference was seen between low and high NCAPH expression patients in PPS time (Figure 4C).

### ***GO functional, KEGG pathway enrichment and PPI network analysis of co-expressed genes of NCAPH***

As shown in Figure 5A, we analyzed the co-expressed genes of NCAPH using three databases: UALCAN, cBioPortal

and GEPIA. The number of co-expressed genes in the three databases were 3,182, 13,480 and 200 separately. One hundred and seventy-five co-expressed genes were in commonly shared. In order to understand the function of these genes, GO functional analysis (cellular component, molecular function and biological process) and KEGG pathway enrichment analysis were performed by using DAVID database (Table 1).

The top ten GO terms were enriched shown in Figure 5B (cellular component), Figure 5C (molecular function) and Figure 5D (biological process). The mainly enriched items for cellular component were chromosome, chromosomal part, intracellular non-membrane-bounded organelle, non-membrane-bounded organelle and spindle. As far as molecular function items, ATP binding, adenylyl nucleotide binding, purine nucleoside binding and nucleoside binding were significantly enriched. The co-expressed genes mainly participated the biological processes of cell cycle, cell cycle phase, M phase, cell cycle process, and mitotic cell cycle. The mainly enriched KEGG pathway items were cell cycle, DNA replication, oocyte meiosis, progesterone-mediated oocyte maturation, and mismatch repair (Figure 5E).

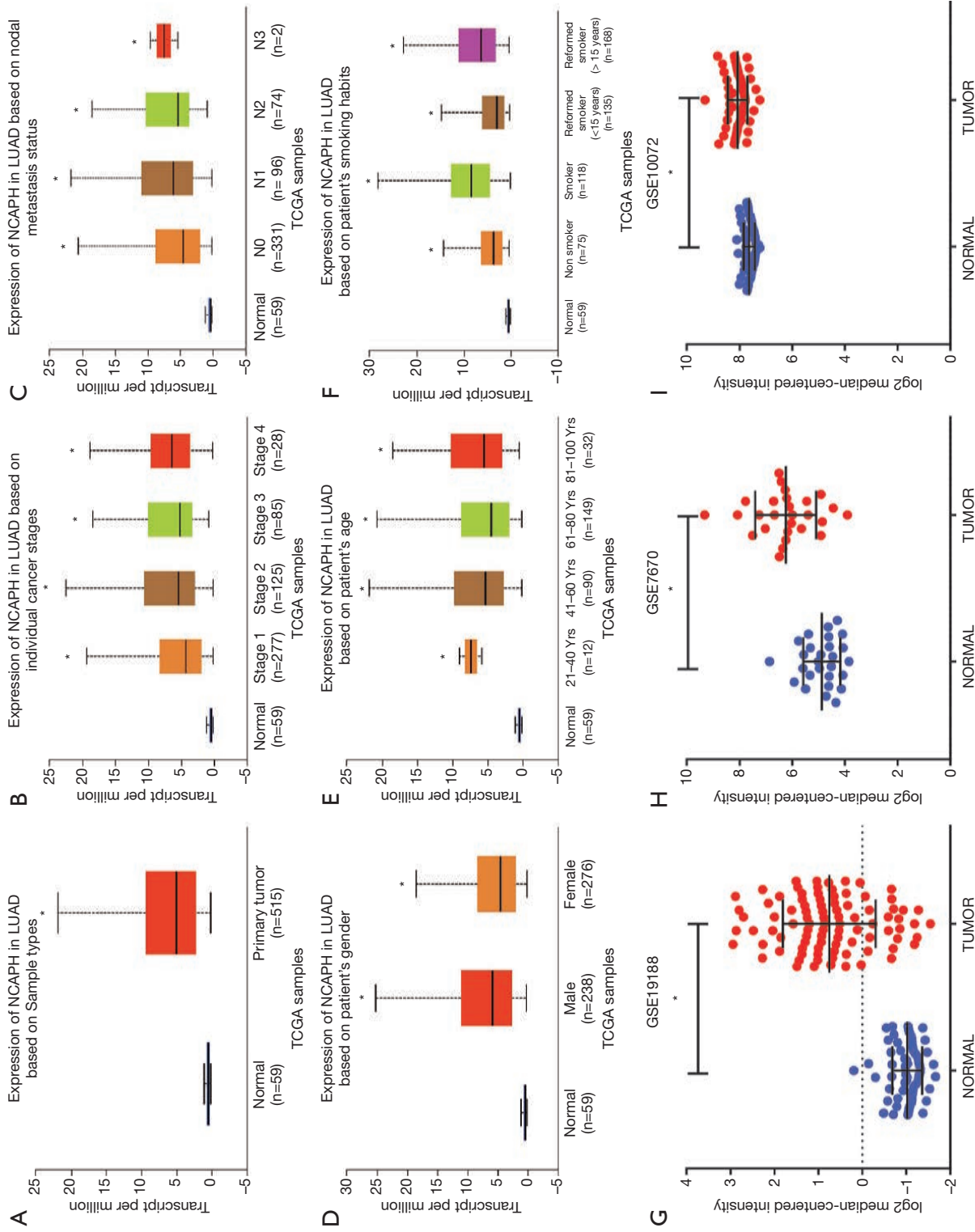
The PPI network analysis was performed by using STRING database and Cytoscape software, containing 175 nodes and 7,840 edges. To find out the key role genes in the network, the cytoHubba app based on Cytoscape software was utilized. Nodes' degree were calculated and ranked by degree from high to low. The top 9 genes were *CDK1*, *BUB1*, *BUB1B*, *CCNB1*, *CCNA2*, *KIF11*, *TOP2A*, *CDC45*, *CDC20* (Figure 5F).

### ***Validation of the prognosis role of co-expression hub genes***

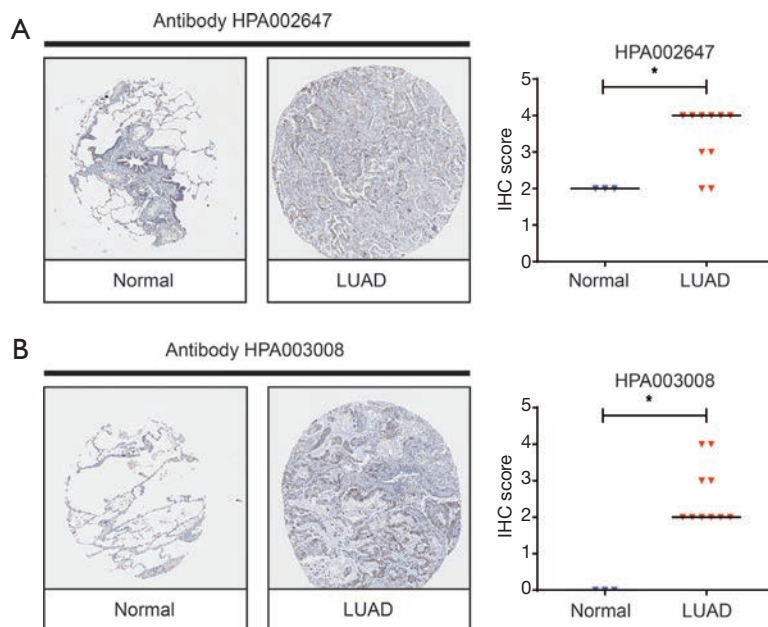
We searched the GEPIA database to furtherly validate the prognosis role of co-expression hub genes. As shown in Figure 6, The top 9 genes, *CDK1*, *BUB1*, *BUB1B*, *CCNB1*, *CCNA2*, *KIF11*, *TOP2A*, *CDC45*, and *CDC20*, acted as onco-gene role in LUAD. Patients with high level of these gene were proved to have worse prognosis compared with that with low level.

### ***Prediction of miRNAs that regulate NCAPH***

To predict miRNAs that regulate NCAPH, TargetScan database was used to find the potential miRNAs. The results contained 1,075 miRNAs with conserved sites or poorly conserved sites. After searching OncomiR database, we found 123 miRNAs with significantly increased expression



**Figure 2** Analysis of NCAPH mRNA based on TCGA and GEO database. (A) The differences of NCAPH mRNA expression in normal lung tissue and lung adenocarcinoma (LUAD) tissue. The analysis of the differences of NCAPH mRNA expression between normal lung tissue and the subtype LUAD tissue divided by tumor stage (B), nodal metastasis status (C), gender (D), age (E), smoking habits (F). Analysis of NCAPH mRNA expression based on GEO database: GSE19188 (G), GSE7670 (H) and GSE10072 (I). \*,  $P < 0.05$ .



**Figure 3** NCAPH protein expression level in normal tissues and Lung adenocarcinoma (LUAD) tissues. (A,B) Based on Human Protein Atlas (HPA) database, the immunohistochemistry (IHC) images were download and then calculated IHC score. (A) antibody HPA002647. (B) antibody HPA003008. \*,  $P < 0.05$ . The blue and red triangles represent IHC scores of normal lung tissue and LUAD, respectively. The magnification of the cell images was 100 $\times$ .

level in LUAD. Moreover, there were 66 miRNAs that had negative correlated relationship with the prognosis in LUAD based on Oncomir. After we drawn the Venn picture of the three miRNA lists, 9 miRNA were found in common: miR-1976, miR-1468-5p, miR-195-3p, miR-490-3p, miR-133b, miR-497-5p, miR-195-5p, miR-125a-5p, miR-500a-3p (Figure 7A). Then, we explored the expression relationship between NCAPH mRNA and each miRNA of the 9 miRNAs. miR-1976, miR-1468-5p, miR-195-3p, miR-490-3p, miR-133b, miR-497-5p, miR-195-5p, and miR-125a-5p had negative correlation with NCAPH mRNA ( $r < 0$ ,  $P < 0.05$ , Figure 7B,C,D,E,F,G,H,I) except miR-500a-3p ( $r > 0$ ,  $P < 0.05$ , Figure 7J). The prognostic values of 8 potential miRNAs in LUAD were shown in Table 2 based on Oncomir database.

#### Potential chemicals that down-regulate NCAPH

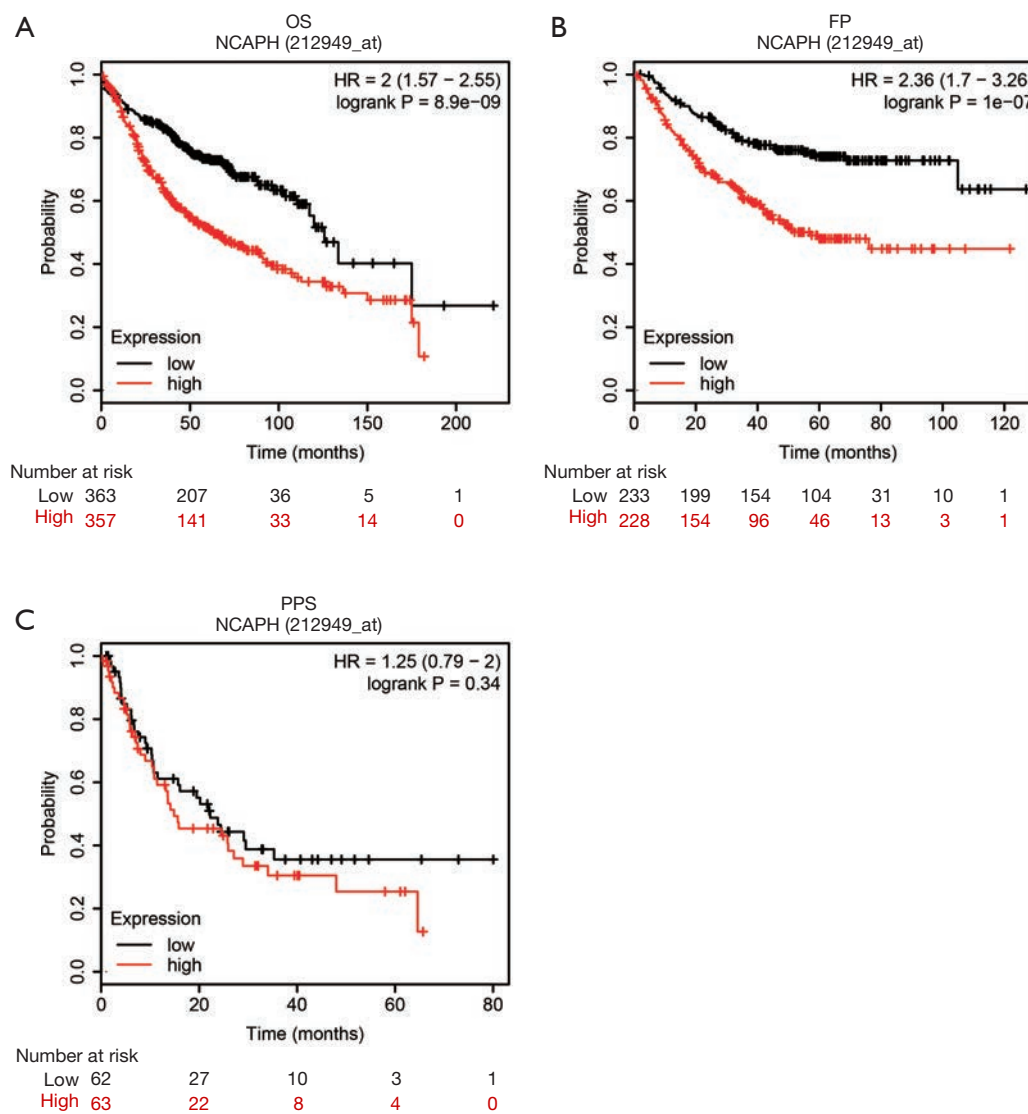
In order to find out chemicals that suppress the NCAPH, we used the CTD database. As a result, 5 chemicals (cyclosporin A, bisphenol A, methyl methanesulfonate, doxorubicin, valproic acid) were identified to decrease the expression of NCAPH mRNA with  $\geq 2$  references. The structure was furtherly downloaded by using PubChem

database (Figure 8).

## Discussion

The knowledge about the prognostic role of NCAPH in LUAD is very poor. In this study, we aimed to demonstrate that NCAPH expression level was elevated in LUAD and predicted poor prognosis. Systematic analysis of the NCAPH expression level in LUAD patients was performed by using TCGA, GEO, and HPA databases from two aspects: mRNA level and protein level (29-31). The co-expression genes enriched analysis suggested that NCAPH may play an important role in cell cycle in LUAD.

Similarly, in prostate cancer, pancreatic cancer and hepatocellular carcinoma, NCAPH is up-regulated and often predicts poor prognosis (10-12). With the help of co-expression network analysis, 9 top enriched co-expressed genes (*CDK1*, *BUB1*, *BUB1B*, *CCNB1*, *CCNA2*, *KIF11*, *TOP2A*, *CDC45*, *CDC20*) were distinguished and considered to play important role in cell cycle. Previous studies showed that *CDK1* and *BUB1* were negatively related with LUAD prognosis (32). *CCNB1*, *CCNA2* and *BUB1B* were regulators in cell cycle and proliferation (33-35). *KIF11* could control bipolar spindle formation and



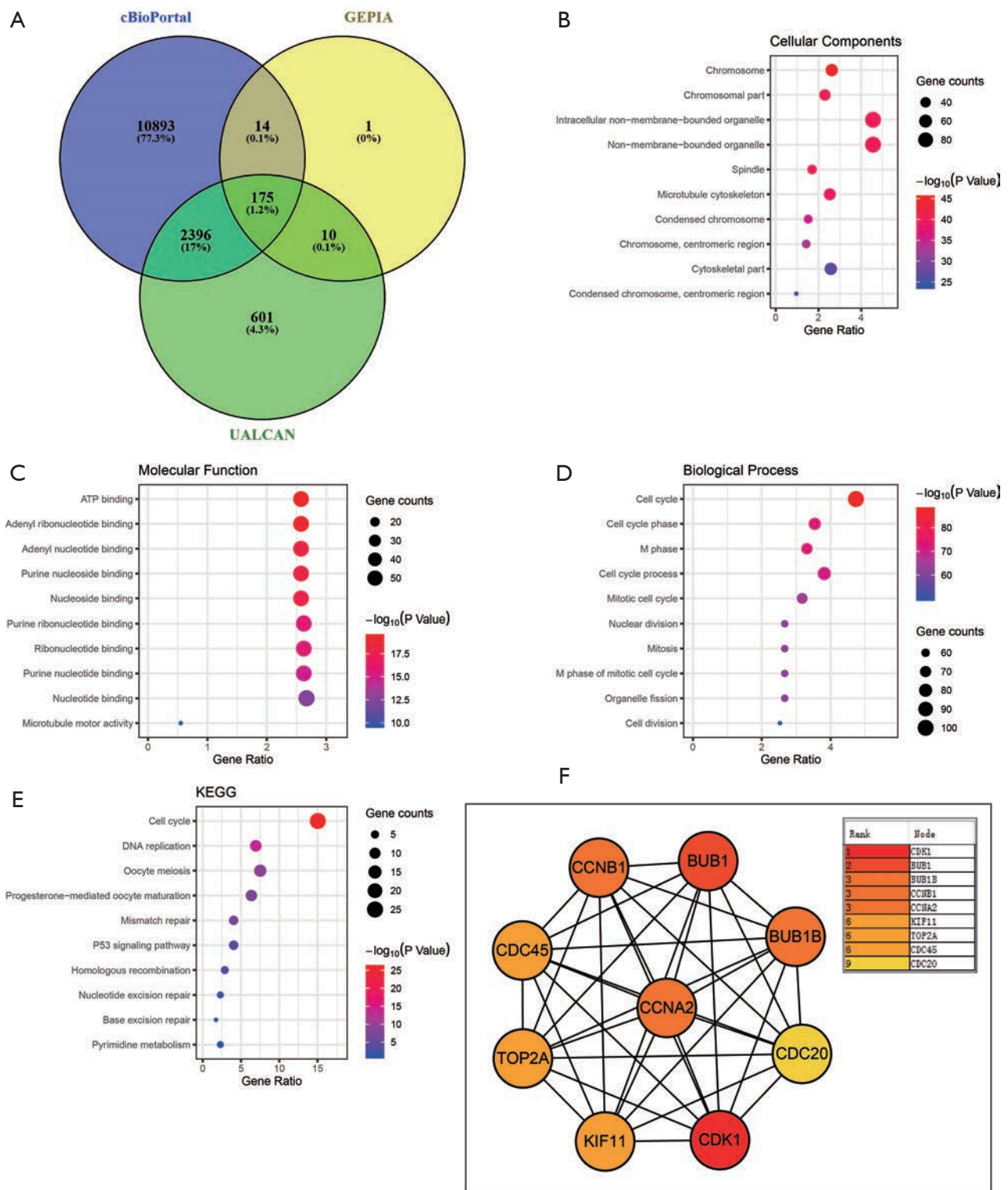
**Figure 4** The prognostic analysis of NCAPH. Kaplan-Meier curves of overall survival time (OS) (A), first progression time (FP) (B) and (post progression survival time) PPS (C).

chromosomal stability (36). TOP2A was considered to be a bad prognosis factor in LUAD (37). These previous studies proved the reliability of our conclusions. We argued that NCAPH probably promoted cell proliferation through cell cycle pathway in LUAD. However, elevated expression of NCAPH meant longer OS time in patients with colon cancer (13). The contradiction may be due to the biological differences of variant tumors.

Why was the NCAPH expression level elevated in LUAD. In order to answer this question, we analyzed the miRNAs in LUAD, which could potentially participate in post-transcriptional regulation of many kinds of proteins.

Previous study showed that miRNAs could sponge 3' UTR of its target mRNA (38). We identified 8 aberrantly down-regulated miRNAs that could potentially inhibit the expression of NCAPH. The correlation analysis of miRNAs and NCAPH mRNA was carried out based on ENCORI, which contained TCGA RNA-Seq data of LUAD. So, the results were more convincing. Among these 8 miRNAs, miR-1976, miR-490-3p, miR-133b, miR-497-5p, miR-195-5p, miR-125a-5p were previously reported as tumor inhibitors in non-small cell lung cancer (39-44). These results are consistent with our findings. Hence, we assume that NCAPH might be down-regulated by these 8 miRNAs.

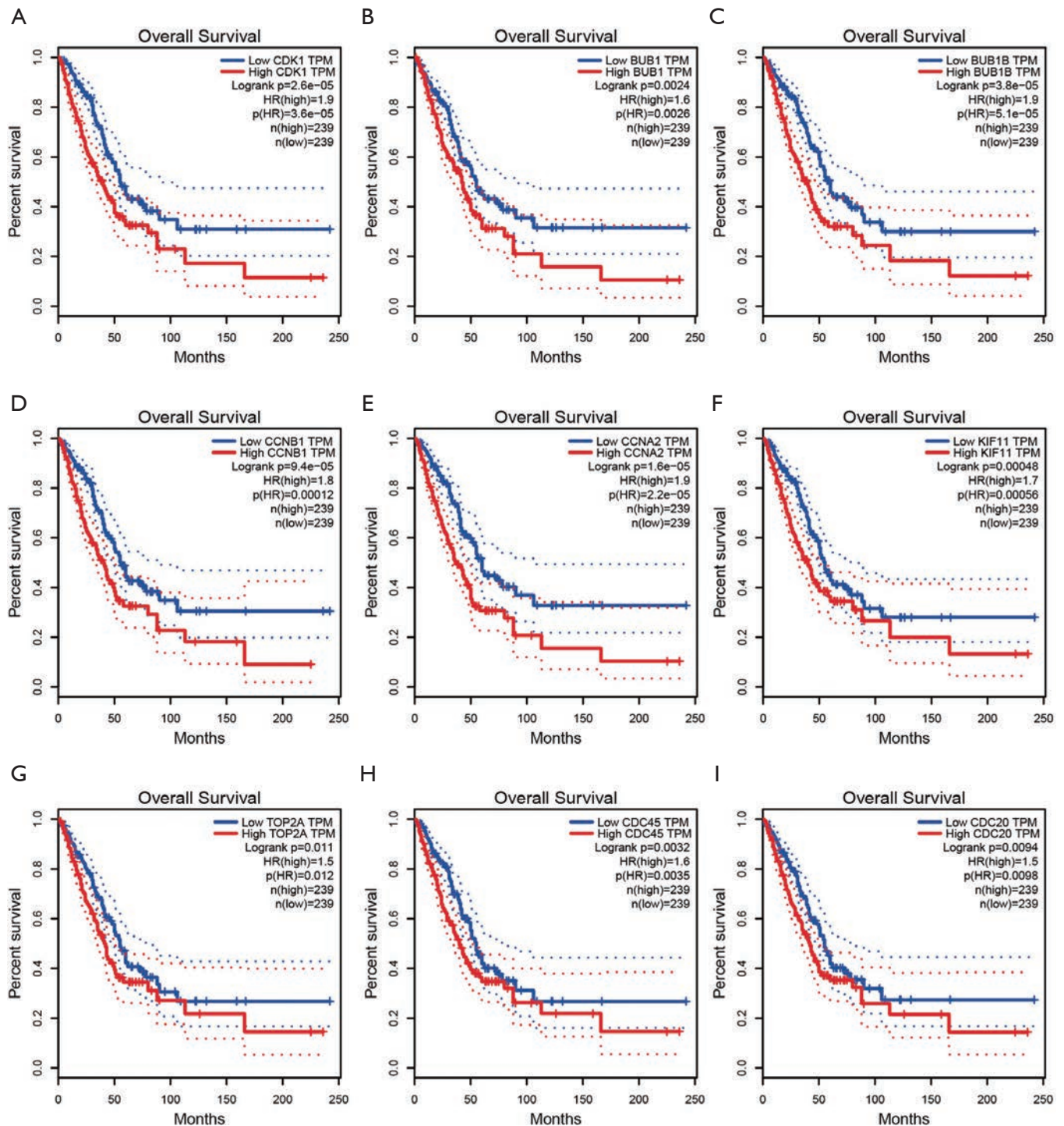




**Figure 5** GO functional, KEGG pathway enrichment and PPI network analysis of co-expressed genes of NCAPH. (A) The Venn picture of co-expressed genes of NCAPH based on GEPIA, UALCAN, and cBioPortal database. Go functional analysis of 175 co-expression genes in three functional groups: cellular component (B), molecular function (C) and biological process (D). (E) KEGG pathway enriched analysis of 175 co-expressed genes. (F) Top 9 co-expressed genes based on the calculation of cytoHubba app.

**Table 1** Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of co-expressed genes of NCAPH in lung adenocarcinoma (LUAD)

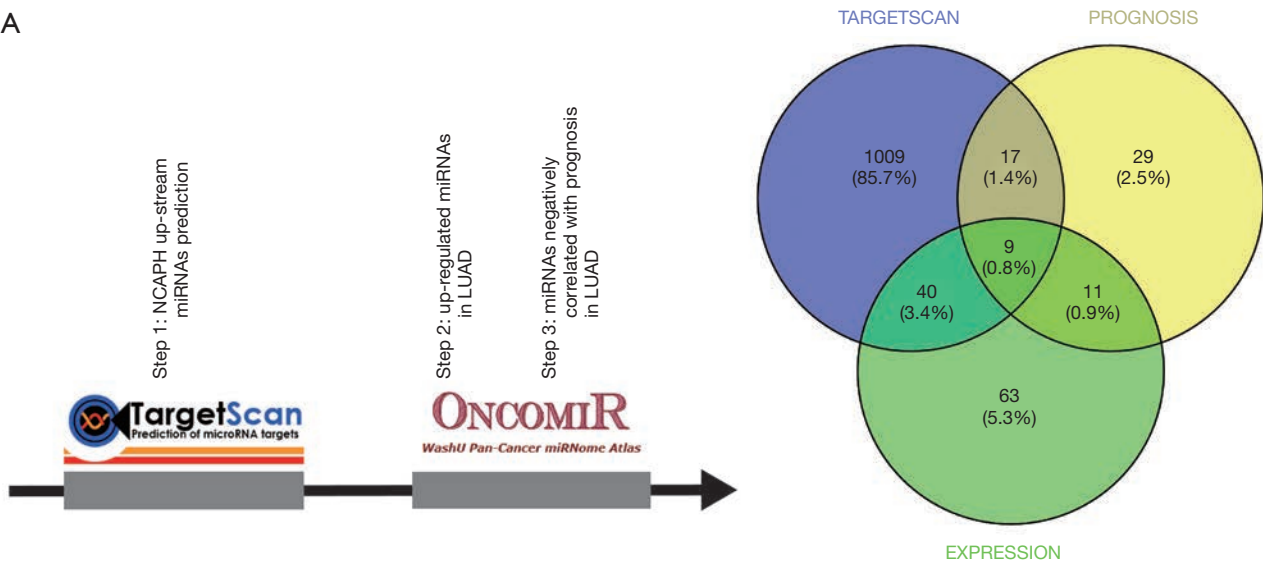
Category	Term	Gene count	P value
GOTERM_CC_FAT	GO:0005694—chromosome	57	5.72E-46
GOTERM_CC_FAT	GO:0044427—chromosomal part	50	1.16E-40
GOTERM_CC_FAT	GO:0043232—intracellular non-membrane-bounded organelle	99	2.27E-40
GOTERM_CC_FAT	GO:0043228—non-membrane-bounded organelle	99	2.27E-40
GOTERM_CC_FAT	GO:0005819—spindle	37	2.65E-40
GOTERM_CC_FAT	GO:0015630—microtubule cytoskeleton	55	3.55E-39
GOTERM_CC_FAT	GO:0000793—condensed chromosome	33	5.77E-36
GOTERM_CC_FAT	GO:0000775—chromosome, centromeric region	31	2.39E-33
GOTERM_CC_FAT	GO:0044430—cytoskeletal part	56	7.13E-28
GOTERM_CC_FAT	GO:0000779—condensed chromosome, centromeric region	21	1.35E-24
GOTERM_MF_FAT	GO:0005524—ATP binding	56	4.19E-20
GOTERM_MF_FAT	GO:0032559—adenyl ribonucleotide binding	56	7.84E-20
GOTERM_MF_FAT	GO:0030554—adenyl nucleotide binding	56	8.73E-19
GOTERM_MF_FAT	GO:0001883—purine nucleoside binding	56	1.75E-18
GOTERM_MF_FAT	GO:0001882—nucleoside binding	56	2.39E-18
GOTERM_MF_FAT	GO:0032555—purine ribonucleotide binding	57	1.69E-16
GOTERM_MF_FAT	GO:0032553—ribonucleotide binding	57	1.69E-16
GOTERM_MF_FAT	GO:0017076—purine nucleotide binding	57	1.53E-15
GOTERM_MF_FAT	GO:0000166—nucleotide binding	58	3.63E-13
GOTERM_MF_FAT	GO:0003777—microtubule motor activity	12	1.96E-10
GOTERM_BP_FAT	GO:0007049—cell cycle	103	1.90E-88
GOTERM_BP_FAT	GO:0022403—cell cycle phase	77	8.97E-74
GOTERM_BP_FAT	GO:0000279—M phase	72	9.33E-74
GOTERM_BP_FAT	GO:0022402—cell cycle process	83	1.35E-71
GOTERM_BP_FAT	GO:0000278—mitotic cell cycle	69	3.24E-65
GOTERM_BP_FAT	GO:0000280—nuclear division	58	3.88E-63
GOTERM_BP_FAT	GO:0007067—mitosis	58	3.88E-63
GOTERM_BP_FAT	GO:0000087—M phase of mitotic cell cycle	58	1.23E-62
GOTERM_BP_FAT	GO:0048285—organelle fission	58	5.02E-62
GOTERM_BP_FAT	GO:0051301—cell division	55	9.61E-51
KEGG_PATHWAY	hsa04110: cell cycle	26	1.41E-26
KEGG_PATHWAY	hsa03030: DNA replication	12	5.00E-14
KEGG_PATHWAY	hsa04114: oocyte meiosis	13	1.44E-09
KEGG_PATHWAY	hsa04914: progesterone-mediated oocyte maturation	11	2.12E-08
KEGG_PATHWAY	hsa03430: mismatch repair	7	1.19E-07
KEGG_PATHWAY	hsa04115: p53 signaling pathway	7	8.78E-05
KEGG_PATHWAY	hsa03440: homologous recombination	5	2.22E-04
KEGG_PATHWAY	hsa03420: nucleotide excision repair	4	0.012179
KEGG_PATHWAY	hsa03410: base excision repair	3	0.056228
KEGG_PATHWAY	hsa00240: pyrimidine metabolism	4	0.08622



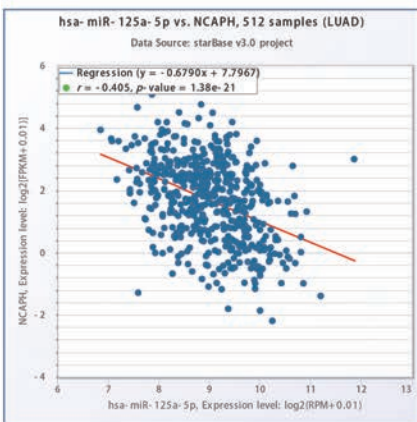
**Figure 6** Prognostic value of co-expressed hub genes in lung adenocarcinoma (LUAD) patients. (A) *CDK1* (B) *BUB1* (C) *BUB1B*, (D) *CCNB1*, (E) *CCNA2*, (F) *KIF11*, (G) *TOP2A*, (H) *CDC45*, (I) *CDC20*.



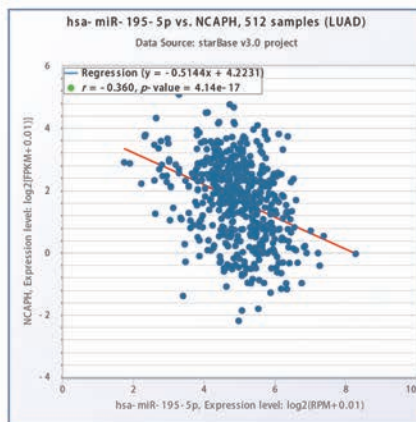
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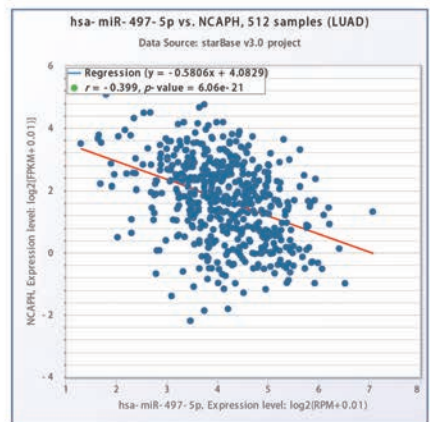
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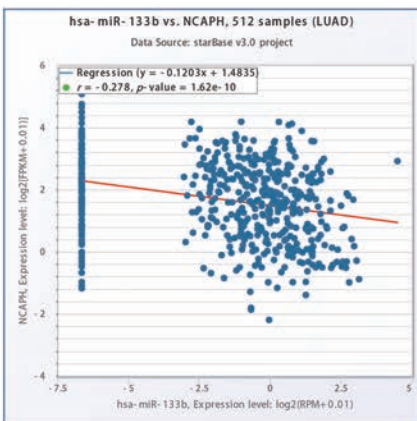
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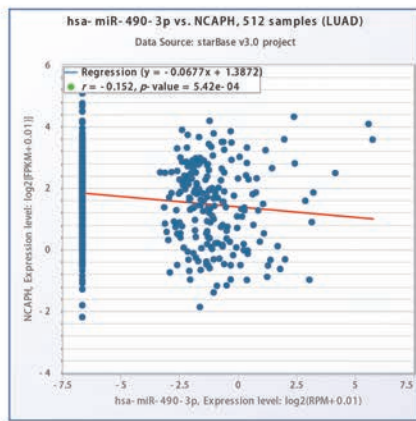
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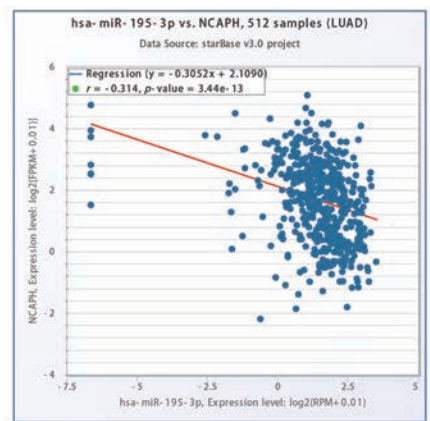
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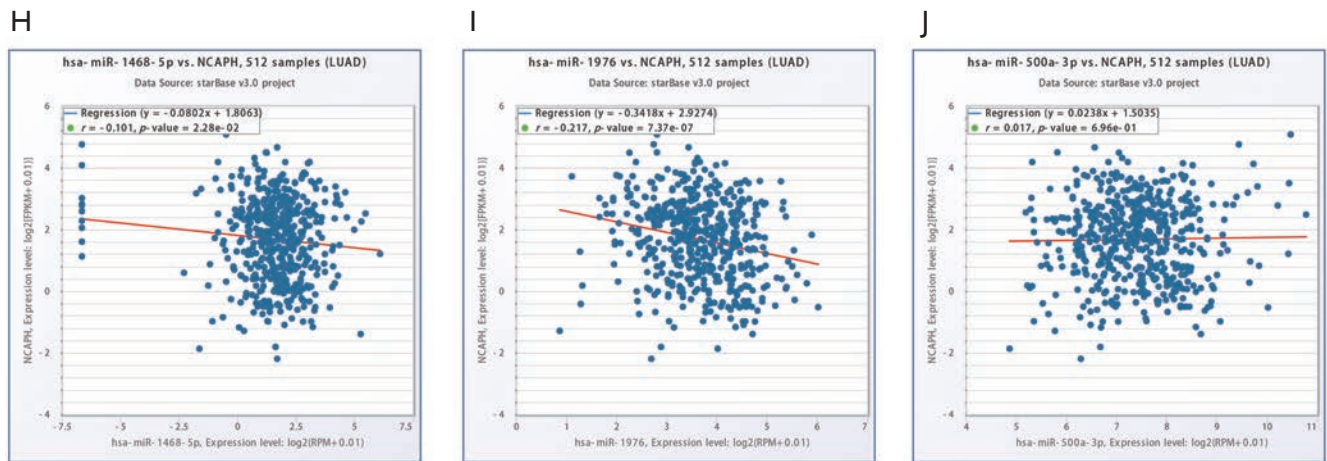
F



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**Figure 7** Prediction of miRNAs that regulate NCAPH. (A) The flowchart and Venn picture of the miRNA prediction process. (B,C,D,E,F,G,H,I,J) The expression correlation between NCAPH mRNA and 9 miRNAs in lung adenocarcinoma (LUAD) patients based on ENCORI database. (B) miR-125a-5p, (C) miR-195-5p, (D) miR-497-5p, (E) miR-133b, (F) miR-490-3p, (G) miR-195-3p, (H) miR-1468-5p, (I) miR-1976, (J) miR-500a-3p.

**Table 2** The prognostic values of 8 potential miRNAs in lung adenocarcinoma (LUAD) analyzed by Oncomir database

miRNA name	Log rank P value	Deceased Log2 mean expression	Living Log2 mean expression	T-test P value
hsa-miR-1976	2.46E-02	3.69	3.95	5.15E-04
hsa-miR-1468-5p	1.16E-03	1.86	2.37	3.51E-04
hsa-miR-195-3p	4.97E-03	1.6	1.87	2.37E-04
hsa-miR-490-3p	1.23E-02	0.1	0.6	5.47E-02
hsa-miR-133b	9.99E-03	0.51	0.7	1.75E-01
hsa-miR-497-5p	1.02E-02	4.23	4.51	7.35E-04
hsa-miR-195-5p	3.48E-02	5.19	5.28	2.98E-01
hsa-miR-125a-5p	2.35E-02	9.09	9.27	1.48E-02

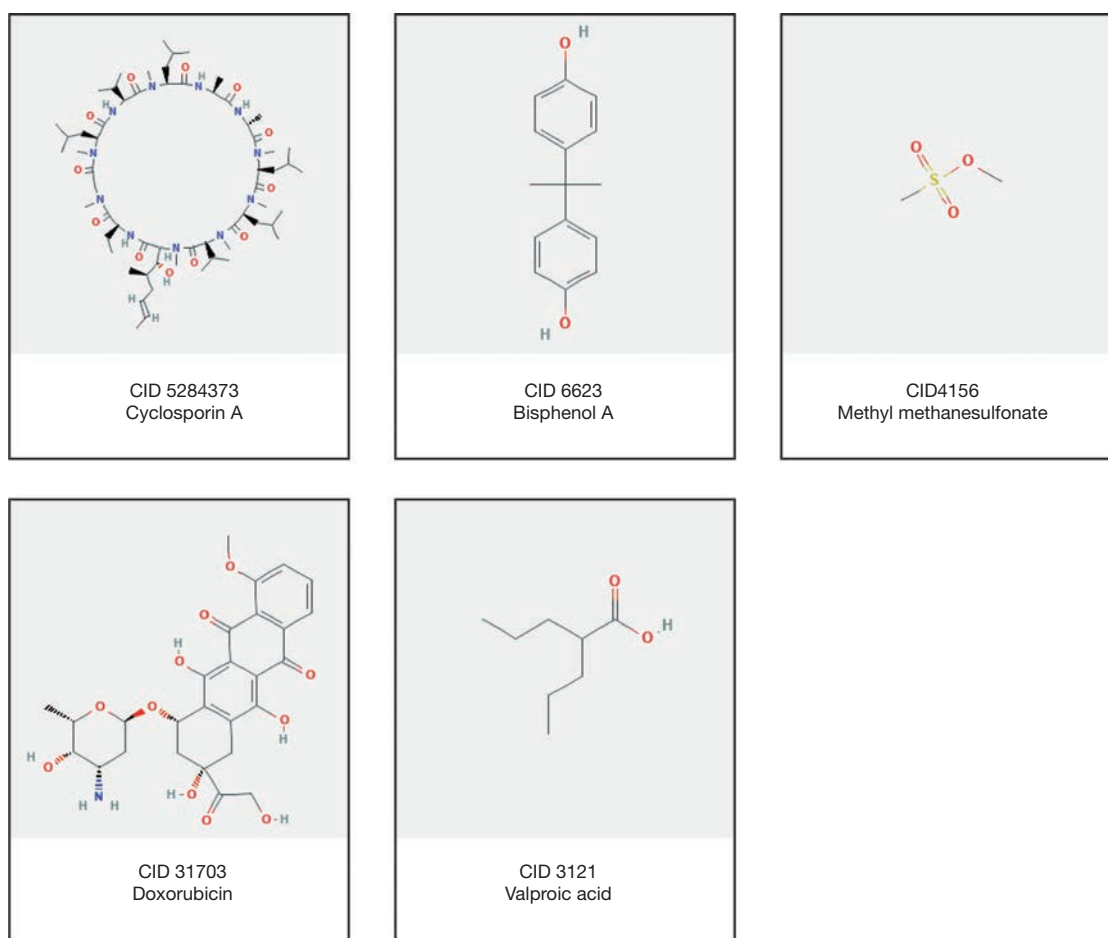
New therapeutic target means a lot to the conquer of cancer. Considering the role of NCAPH as a potential therapeutic target, the CTD database was utilized for finding the chemicals which could down-regulate the NCAPH expression level. Five kinds of chemicals were identified based on previous studies in the database. The problem that whether these chemicals could inhibit the LUAD would be answered with our next experiments.

Despite the big data analysis was used in this study, there are still some limitations: Firstly, lack of the validation in clinical LUAD samples. Secondly, the expression levels of proteins are regulated by many factors, such as promoter

methylation, transcription factor and ubiquitination (45,46). These factors were not analyzed in our study. Thirdly, the onco-gene role of NCAPH was not testified invitro and *in vivo*. These shortcomings will be solved in the next experiment.

## Conclusions

In conclusion, we propose that NCAPH expression in LUAD is a poor prognostic indicator. It may play an important role in cell cycle. In the near future, it may be a potential therapeutic target of LUAD.



**Figure 8** Structure of five chemicals that could decrease the NCAPH.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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