NCOA4 is associated with cancer stem cell markers and acts as a prognostic biomarker for immune infiltration- related lung adenocarcinoma patients

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Research Article

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Abstract

Lung adenocarcinoma is a cancer with a high mortality rate and a low survival rate. Ferroptosis can play an important role in anti-tumor immunity and tumor suppression. NCOA4, as a selective cargo receptor for ferritin autophagy turnover, is very important for Ferroptosis. However, NCOA4 used as a biomarker for the diagnosis, prognosis and treatment of lung adenocarcinoma remains unclean. Therefore, we use ONCOMINE, Tumor Immune Estimation Resource, UALCAN, GEPIA, TNMplot, Human Protein Atlas, Kaplan-Meier plotter, OncoLnc, GeneMANIA, STRING, LinkedOmics database to determine the relationship between NCOA4 expression, prognosis and immunoprecipitation in lung adenocarcinoma. Finally, we used OCLR to calculate mRNA expression-based stemness index of LUAD patient's data in TCGA and analyzed the correlation between NCOA4 and markers of lung cancer stem cells. We obtained the low expression of NCOA4 in lung adenocarcinoma. Moreover, low expression of NCOA4 is positively correlated with poor overall lifetime. Studies based on the TIMER database show that NCOA4 has significant positive correlations with a variety of tumor immune cells, in particular CD8 + T cells, macrophages cells, neutrophil cells, and dendritic cells. Furthermore, we used the LinkedOmics database to evaluate gene co-expression of NCOA4 in LUAD and to investigate their role in tumor immunity and ferroptosis. And we found that NCOA4 is closely related to surface markers of lung cancer stem cells. Therefore, NCOA4 may be a biomarker for predicting the survival and diagnosis of LUAD.

Introduction

Lung cancer is an important cause of cancer death, with an estimated 13%probability of lung cancer being diagnosed in men and 12% among women, ranking second among all cancers[1]. Lung cancer mainly has two subtypes of non-small cell lung cancer and small cell lung cancer, of which non-small cell lung cancer accounts for about 80%of lung cancer cases, divided into lung adenocarcinoma and lung scale cancer two types[2]. Lung adenocarcinoma has a high mortality rate, killing more than 10 million people worldwide each year. Cancer cells can be quickly transferred to different organs in a very short period of time, and traditional treatments for radiotherapy and chemotherapy have little effect on lung adenocarcinoma[3]. In recent years, cancer therapy has made some progress, such as targeted therapy and immunotherapy[4, 5]. Therefore, it is important to find reliable biomarkers to diagnose and predict lung adenocarcinoma, which will contribute to the effective treatment of lung adenocarcinoma.

Ferroptosis is a new type of cell death, accompanied by a large amount of iron accumulation and lipid peroxidation during cell death, characterized by increased mitochondrial membrane density and cell volume contraction, which are different from other morphological, biochemical and gene-regulated cell deaths[6, 7]. In recent years, induced ferroptosis has become a promising alternative treatment. Ferroptosis plays a possible pathogenic role in cancer, neurodegeneration, and organ dysfunction[8, 9]. Therefore, inducing ferroptosis may trigger the death of cancer cells, can effectively to target those resistant to traditional therapies of malignant tumors[10, 11]. Today, not only are there drugs that induce ferroptosis, but many genes have been identified as modulators or markers of ferroptosis. These findings will boost the prospects of ferroptosis in cancer treatment[9, 10, 12].

NCOA4 is a selective cargo receptor that regulates the autophagic degradation of the complex ferritin stored in cell sol iron during the iron oxidation process[13]. Autophagy is a mechanism that regulates protein and cell degradation and it plays an indispensable role in the body's balance[14]. Autophagy is not only associated with survival, development, and balance, but also with cancer, neurodegeneration, and microbial infections[15]. NCOA4 has been identified as a selective cargo receptor for autophagy turnover of ferritin and is important for Ferroptosis [16]. Moreover, NCOA4-mediated ferritinophagy can support ferroptosis by controlling iron balance in cells[17]. In addition, studies have confirmed that NCOA4 is closely related to tumorigenesis[18]. NCOA4 can serve as a reliable prognostic biomarker for clear cell renal cell carcinoma and may be a potential therapeutic target[19]. We also identified a new 15-gene signature associated with Ferroptosis, including NCOA4, that not only predicts the prognosis of lung adenocarcinoma, but also provides new therapies for lung adenocarcinoma populations[20]. Therefore, we urgently explore the detailed and in-depth relationship between NCOA4 and lung adenocarcinoma, providing a potential theory for the prognosis and treatment of lung adenocarcinoma patients.

Cancer stem cells are groups of cells in tumors that have the ability to self-renew. Studies have shown that targeting iron death may specifically kill cancer stem cells. Salinomycin targets breast CSCs by driving ferroptosis-induced cell death[21]. Erastin, an inhibitor of SLC7A11, is highly cytotoxic to colorectal CSCs, reducing chemotherapeutic resistance of colorectal cancer stem cells and making them more sensitive to iron death[22]. As a marker of iron autophagy, down-regulated expression of NCOA4 may promote the uptake of lysosomal ferritin and accumulation of intracellular free iron, leading to cell iron death[23]. CD44 is a multifunctional cell surface glycoprotein involved in proliferation, differentiation, migration, angiogenesis and other cellular processes, and is considered as a marker of CSCs in lung cancer[24, 25]. CD166, also known as ALCAM, is an indicator of poor prognosis in some cancers and is also considered to be a marker of CSCs in lung cancer[26, 27]. CD90, a glycosylphosphatidylinositol anchored glycoprotein, also known as TGY1, has been confirmed to be expressed in mouse breast CSCs and primary advanced glioma CSCs, and is also considered as a CSCs marker in lung cancer[28]. Abnormal expression of NCOA4 may lead to cell iron death, thus targeting lung adenocarcinoma stem cells and playing an important role in targeted lung adenocarcinoma therapy.

However, the expressive and prognostic potential of NCOA4 in lung adenocarcinoma is not yet known. The link between NCOA4 and immune immersion in lung adenocarcinoma has not been widely studied. Therefore, here we studied the levels of mRNA and protein expression in NCOA4 in lung adenocarcinoma and tested the prognostic value of NNA4. We built the PPI network of NCOA4 to explore the mechanisms and functions of NCOA4. In addition, we analyzed the correlation between NCOA4 and tumor immuno-immersion cells. Finally, we analyzed the mRNA expression-based stemness index for different expressions of NCOA4 in lung adenocarcinoma and predicted the association between NCOA4 and surface markers of lung cancer stem cells. Our results demonstrate the important role of NCOA4 in lung adenocarcinoma, providing a potential link between NCOA4 and the immune immersion of lung adenocarcinoma and its potential mechanisms.

Materials And Methods

Oncomine database

The Oncomine (https://www.oncomine.org/) database, which includes 715 datasets and 86,733 samples[29], was utilized to analyze the mRNA levels of NCOA4 in lung cancer and normal tissues. Our search was performed based on the following criteria: P-value < 0.05, fold change < 1.5, and gene ranking all.

GEPIA database

GEPIA (http://gepia.cancer-pku.cn/), a mining online database, pulls data from the UCSC Xena server[30]. We utilized the GEPIA database to investigate NCOA4 mRNA expression in lung cancer. In addition, we assessed the association of NCOA4 with LUNG cancer stem cells CD44,CD166, and CD90.

UALCAN database

The UALCAN (http://ualcan.path.uab.edu) database provides comprehensive cancer transcriptome and clinical patient data (pulled from TCGA) [31]. We evaluated the expression level of NCOA4 to compare it not only between lung cancer and corresponding normal tissues but also across various subgroups stratified by sex, pathological stage, tumor grade and other clinicopathological parameters.

TNMplot database

TNMplot (https://tnmplot.com/analysis/) is the largest currently available transcriptomic cancer database by utliaing multiple RNA-seq and microarray. And it is a database for the comparison of gene expression in malignant, normal and metastatic tissues[32]. We used TNMplot database to evaluate NCOA4 expression in LUAD.

Human Protein Atlas database

Human Protein Atlas (https://www.proteinatlas.org/) is a new publicly available database containing, the first version, ~400,000 high resolution images corresponding to more than 700 antibodies toward human proteins[33]. We used the HPA database for immunohistochemical analysis.

TIMER database

TIMER (https://cistrome.shinyapps.io/timer/) is an interactive and user-friendly online tool that can be used to systematically evaluate the expression of gene sets related to infiltrating immune cells in data from TCGA[34]. In the present study, the connection between NCOA4 expression and immune cell infiltration in LUAD was analyzed. Moreover, associations between NCOA4 and gene markers of diverse tumor-infiltrating immune cells were investigated through TIMER.

Kaplan-Meier plotter database

The relationships between the expression level of NCOA4 and prognosis (i.e., overall survival (OS), and post-progression survival (PPS)) of cancer patients were examined with Kaplan-Meier plotter[35]. In addition, the prognostic potential of NCOA4 based on multiple clinicopathological features was also analyzed with Kaplan–Meier plotter (http://kmplot.com/analysis/).

OncoLnc database

OncoLnc (http://www.oncolnc.org/) contains survival data for 8,647 patients from 21 cancer studies performed by The Cancer Genome Atlas (TCGA), along with RNA-SEQ expression for mRNAs and miRNAs from TCGA, and lncRNA expression from MiTranscriptome beta[36]. In this study, the prognostic potential of NCOA4 was further analyzed using OncoLnc database.

Interaction network analysis

GeneMANIA (http://genemaia.org) website is used to construct the PPI network through a large number of correlation data including physical interactions, co-expression, predicted, co-localization, pathway, genetic interactions, and shared protein domains [37]. In this study, the GeneMANIA online database was utilized to generate the NCOA4 interaction network. STRING (http://www.oncolnc.org/) is an online database for predicting protein-protein interactions [38]. The STRING database was utilized to generate a PPI network of NCOA4.

LinkedOmics database

LinkedOmics (http://www.linkedomics.org/) is a web-based platform for analyzing 32 TCGA cancer-associated multi-dimensional datasets[39]. We use LinkedOmics database to analysis NCOA4 co-expression, presenting in volcano plots, heat maps.

A machine learning algorithm and mRNA expression-based stemness index (mRNAsi) of LUAD patients' data in TCGA

RNA-sequencing expression (level 3) profiles and corresponding clinical information for NCOA4 were downloaded from the TCGA dataset. Use the OCLR algorithm to calculate mRNAsi which constructed by Malta et al. . Based on the mRNA expression signature, the gene expression profile contains 11,774 genes. We used the same Spearman correlation (RNA expression data)[40, 41]. The minimum value was subtracted, and the result was divided by the maximum maps the dryness index to the range [0,1]. We used this method to analyzed the mRNA expression-based stemness index for different expressions of NCOA4 in lung adenocarcinoma.

Results

mRNA and protein expression of NCOA4

We assessed NCOA4 expression levels using the Oncomine database. The NCOA4 expression levels of Brain and CNS cancer, Leukemia, Other cancer, Pancreatic cancer, Sarcoma were significant increased than those normal tissues. However, The NCOA4 expression levels of Breast cancer, Leukemia, Lung cancer, Lymphoma and Other cancer were obviously decreased compared with those normal tissues. (Figure1, A). And then we used the TIMER database to analyze the expression of NCOA4 in more human cancers. The result showed that NCOA4 expression levels differences in tumor tissue and adjacent normal tissue (Figure1, B): NCOA4 expression in BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma), COAD (Colon adenocarcinoma), KICH (Kidney Chromophobe), KIRP (Kidney renal papillary cell carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), READ (Rectum adenocarcinoma), SKCM (Skin Cutaneous Melanoma), THCA (Thyroid carcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) were lower than those of neighboring tissues. In addition, Data mining using the GEPIA and UALCAN, TNM plot databases was further confirmed that NCOA4 expression is decreased in LUAD (Figure1, C-F). We also used the HPA database for immunohistochemical analysis (Figure1, G). The protein expression level of NCOA4 was distinctly decreased in lung adenocarcinoma.

Relationship between expression of NCOA4 and clinicopathological characteristics of LUAD

In order to explore the reasons for the low expression of NCOA4 in lung adenocarcinoma, the UALCAN database was used to further analyze the correlation between NCOA4 expression and clinical information. In terms of patient's age, significant low expression of NCOA4 was observed in 41-100 years (Figure2, A). As shown in Figure 2B, mining of the UALCAN database results suggested that NCOA4 expression was decreased in males and females. For the cancer stage, NCOA4 expression level was significantly decreased in stage 1, 2, 3,4 (Figure2, C). At the same time, analysis of patient's race, TP53 muation status, patient's smoking habits showed that the expression level of NCOA4 in patients with lung adenocarcinoma decreased relative to normal samples (Figure2, D-F).

Prognostic analysis of NCOA4 in LUAD

Next, we applied to Kaplan-Meier plotter to explore the correlation between NCOA4 expression and the prognosis of LUAD patient. As a result, patients of LUAD with high NCOA4 level had better overall survival rates than patients with low NCOA4 level (Figure3, A). In the meantime, low NCOA4 expression was correlated with poor PPS and poor FP as shown in Figure3B, C. In addition, the prognostic potential of NCOA4 was further analyzed using OncoLnc database (Figure3).

Correlation between expression of NCOA4 and infiltrating immune cells

Then, we explored the correlation between NCOA4 expression and six major tumor-infiltrating immune cells in the TIMER database. The results showed that NCOA4 expression levels were significantly associated with CD8+T cells (r=0.365, p=8.41e-17), macrophages cells (r=0.386, p=1.19e-18), neutrophil cells (r=0.329, p=1.09e-13), dendritic cells (r=0.336, p=2.30e-14) in LUAD (Figure4, A). The expression level of NCOA4 was remarkably positively correlated with CD8+T cells, macrophages cells, neutrophil cells and dendritic cells, indicating that the expression level of NCOA4 was positively related to lung

adenocarcinoma immune infiltration. We also explored the difference of immune cells in different groups of samples through CIBERSORT. As shown in Figure4B, NCOA4 levels were found to be correlated with infiltration by sixteen types of tumor-infiltrating cells, including memory B cell, plasma B cell, resting memory CD4+T cell, activated memory CD4+T cell, follicular helper T cell, Tregs, gamma delta T cell, resting NK cell, Monocyte, Macrophage M0, Macrophage M1, resting Myeloid dendritic cell, activated Myeloid dendritic cell, activated Mast cell, Eosinophil, Neutrophil.

Relationship between NCOA4 expression level and different immune marker sets

Based on a set of immune markers in LUAD, we analyzed the link between NCOA4 expression and immune markers through the TIMER database (Table1). NCOA4 expression was significantly correlated with the levels of most markers in different types of immune cells in lung adenocarcinoma. It is interesting to note that NCOA4 expression showed a strong positive association with M2 macrophages and TAMs immune marker genes. After adjustment for tumor purity, we found a significant correlation between NCOA4 expression and markers of M2 macrophages and TAMs. Expression of CCL18, CD163, MS4A4A, and MRC1 of M2 macrophages and CCL2, CD86, CCR5and CD80 of TAMs showed a significantly positive correlation with NCOA4 expression ($P<0.001$). However, M1 macrophage markers, such as IRF5, ARG2 and PTGS2 showed a weak or no correlation with NCOA4 expression. M1 macrophage markers, such as CXCL10 and NOS2 show a correlation with NCOA4 expression. These findings elucidated that NCOA4 expression was correlated with immune infiltration, and it might serve as a potential immunotherapeutic target for LUAD treatment.

Prognostic value of NCOA4 from immune cells in LUAD

To investigate whether the expression of NCOA4 affects the prognosis of LUAD patients by directly influencing the infiltration of immune cells, we used the KM plotter database to analyze the prognosis of NCOA4 expression in different subgroups of immune cells in LUAD. As shown in Figure5, low expression of NCOA4 in enriched B cells, enrich CD8+T cells, enriched macrophages, decreased natural killer T cells, decreased type 1 T helper cells, enriched type 2 T helper cells cohorts in LUAD was associated with poor OS. Furthermore, there was no significant correlation between NCOA4 expression and decreased CD8+T cells, decreased macrophages, enriched natural killer T cells, enriched type 1 T helper cells, decreased type 2 T helper cells cohorts in LUAD.

Network construction of NCOA4 interacting genes and proteins.

We used the GeneMANIA database to constructe the gene-gene interaction network of NCOA4. There are 20 surrounding nodes represent genes associated with NCOA4. The middle node represents NCOA4(Figure6, A). The three genes most significantly associated with NCOA4 are PPARA, AR, RXRA. Then, in order to further investigate the biological role of NCOA4, we used the STRING database to constructe a PPI network.

Construction of NCoA4 co-expression network and GO, KEGG enrichment analysis.

In order to understand the biological function of NCOA4 in lung adenocarcinoma, we used the LinkedOmics database to build a co-expression network of NCOA4. As shown in Figure7A, 7576genes (red dots) are positively related to NCOA4 and 10520 genes (green dots) are negatively related to NCOA4(P<0.05). As shown in Figure 7B-C, the first 50 positive and negative genes associated with NCOA4 are shown as heat maps. NCOA4 expression is presented with genes strong positive correlation such as SPRR3(positive correlation, r=0.895, p=0.0348), MFSD1(r= 0.848, p=4.16e-29), CTSS (r=0.812, p=7.71e-25), but negative correlation with genes such as PHC1(negative correlation, r=-0.714, p=5.25e-17), DLG5(r=-0.703, p=2.48e-16), CEP164(r=-0.702, p=2.76e-16). In addition, we used the LinkedOmics to perform a rich analysis based on the NCOA4 co-expression gene network. As shown Figure7D-G, at the level of biological process, NCOA4 co-expression is rich in the positive regulation of neutrophil mediated immunity, translation initiation, antigen processing and presentation and so on. At the cell component level, NCOA4 co-expression is mainly rich in ribosome, primary lysosome, vesicle lumen, and at the molecular function level, NCOA4 co-expression is mainly rich in structural constituent of ribosome, electron transfer activity, oxidoreductase activity, acting on NAD(P)H. At the KEGG pathway, NCOA4 co-expression is mainly rich in Ribosome, Lysosome, Ferroptosis.

Correlation between NCAO4 and surface markers of lung cancer stem cells.

Tumor stem cells play a key role in tumorigenesis, development and metastasis. We first used machine learning to evaluate the stemness index of lung adenocarcinoma samples. We found that the low expression group of NCOA4 had a higher mRNAsi(Figure8, D). In addition, we used GEPIA to predict the correlation between NCOA4 and lung cancer stem cell surface markers. We found that NCOA4 was significantly positively correlated with CD44, CD166(ALCAM), and CD90(THY1)(Figure8, A-C). These results suggest that NCOA4 may interact with lung cancer stem cell surface markers, play an important role in targeted treatment of lung adenocarcinoma, and can be used as a reliable biomarker of lung adenocarcinoma.

Discussion

Lung adenocarcinoma is a highly fatal cancer with a high incidence. Even though screening, diagnosis and traditional treatment have made some progress, the results are not satisfactory. However, Ferroptosis is a cell death mechanism dependent on iron metabolism, which can be used as an alternative strategy to provide an effective option for treatment[42]. Studies have shown that ferroptosis can play an important role in antitumor immunity and tumor inhibition[43]. The expression of FSP1 is associated with iron resistance in lung cancer cell lines[44]. SLC7A11 inhibits the development of NSCLC by ferroptosis through regulation of XAV939[45]. NCOA4-mediated ferritinophagy regulates susceptibility to ferroptosis. However, these findings suggest the treatment of ferroptosis in lung cancer, but the underlying mechanism of ferroptosis and genes in lung adenocarcinoma remains unclear.

What's more, NCOA4 acts as a selective autophagy cargo receptor, first by binding to FTH1 to autophagy ferritin turnover, and then NCOA4 transfers NCOA4 -ferritin complex to autosomes, mediating the

degradation of ferritin, and converting ferritin bound iron to free iron, thereby inducing ferritin poisoning[46]. Furthermore, NCOA4 deletion leads to a cellular disorder of ferroptosis by eliminating intracellular accumulation of free iron, glutathione, and reactive oxygen species (ROS), and is strongly associated with the occurrence and progression of various cancers, such as prostate, ovarian, and breast cancers[47].

Therefore, we investigated the role of NCOA4 expression in tumorigenesis and progression based on various online databases. First, we used differential expression to study the expression of NCOA4 in various tumors. The expression level of NCOA4 is down-regulated in lung adenocarcinoma. We used the UALCAN database to predict the expression level of NCOA4 in lung adenocarcinoma based on clinicopathological features. NCOA4 expression was decreased at all tumor stages and was strongly associated with the patient's smoking habits. In addition, we also analyzed the effect of NCOA4 expression on the survival rate of cancer patients. Low expression of NCOA4 was significantly associated with poor prognosis in patients with lung adenocarcinoma.

Lung tissue contains a large number of immune cells, providing a powerful defense against foreign particles and microorganisms[48]. More importantly, in the present study, GO and KEGG results indicate that NCOA4 is associated with multiple signaling pathways, including the immune response. Therefore, we used the TIMER database to explore the correlation between the expression of NCOA4 and the six immersive immune cells in LUAD. The results showed that the expression of NCOA4 was strongly positively correlated with CD8-T cells, macrophages, dendritic cells, and neutrophils. The results of the CIBERSORT database also show that the expression of NCOA4 is related to Immune cells such as B cell, T cell, NK cell, Monocyte, Macrophage. In addition, the expression of NCOA4 is related to the genetic markers of M1 macrophages and M2 macrophages. The results showed that M2 macrophage gene markers were strongly correlated with NCOA4 expression, TAM markers were moderately correlated with NCOA4 expression, and M1 markers were weakly correlated with NCOA4 expression. Similarly, the expression of NCOA4 was significantly correlated with the regulation of a variety of T-assisted cell markers. According to the literature, we understand that TAMs are the most abundant cell in TME osmotic immunosuppressive cells and plays a key role in influencing the efficacy of anti-tumor immunotherapy[49]. Moreover, TAMs are highly plastic and heterogeneous in solid tumor. Th1 cytokines such as liposaccharides (LPS), interferon-γ (IFN-γ) and tumor necrosis factors - α induce macrophages into M1-like phenotypes and play a role in antitumor inflammation[50]. In contrast, THE (M2-like), polarized by IL-4 and IL-13, plays the opposite immunosuppressive and pro-tumor function in TME[51]. Sum up, the close relationship between the expression of NCOA4 and immune cells may explain the potential of NCOA4 in regulating tumor-related immune cells. These findings suggest that NCOA4 plays an important role in the recruitment and regulation of immune cells inundated with lung adenocarcinoma cells. In addition, mRNAsi of LUAD patient data in TCGA was calculated by OCLR, and we found that low expression of NCOA4 was associated with higher mRNAsi. Moreover, NCOA4 was closely positively correlated with CD44, CD166 and CD90 markers of lung cancer stem cells. These results also suggest that NCOA4, which is closely associated with ferroptosis, may interact with lung cancer stem cell

markers. NCOA4 may target ferroptosis and specifically kill lung adenocarcinoma stem cells, which may serve as a reliable biomarker for tumor therapy.

At present, how NCOA4 affects the prognosis of LUAD and the function of NCOA4 in LUAD has not been fully elaborated. Our study provided new insights into the possible role of NCOA4 in LUAD. However, there are some limitations. All the data analyzed were retrospective and from multiple public datasets, which were not verified by our own experiments. More effort is needed to explore the detailed mechanisms of NCOA4 in LUAD.

Conclusions

In conclusion, we used multiple online databases to perform a comprehensive analysis between NCOA4 and LUAD and identified that low NCOA4 expression in LUAD predicted adverse prognosis. What's more, we found NCOA4 expression was positively associated with the infiltration level of immune cells, particularly macrophages. Moreover, mRNAsi of NCOA4 low expression was higher. NCOA4 was positively correlated with lung cancer stem cell markers CD44, CD166 and CD90.We identified NCOA4 as a novel diagnostic and prognostic biomarker and also a new immunotherapeutic target for LUAD.

Declarations

Author Contributions: Lianxiang Luo conceived and designed the study. Manshan Li performed data mining and analysis. Manshan Li and Lianxiang Luo wrote the manuscript. Lianxiang Luo reviewed the paper and provided comments. All authors contributed to the article and approved the submitted version.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
2. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553(7689):446–454.

3. Author Correction: Comprehensive molecular profiling of lung adenocarcinoma. Nature. 2018;559(7715):E12.
4. Low JL, Walsh RJ, Ang Y, Chan G, Soo RA. The evolving immuno-oncology landscape in advanced lung cancer: first-line treatment of non-small cell lung cancer. Ther Adv Med Oncol. 2019;11(1758835919870360.
5. Li XT, Yang JJ, Wu YL, Hou J. Toward innovative combinational immunotherapy: A systems biology perspective. Cancer Treat Rev. 2018;68(1–8.
6. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. Cell Death Dis. 2020;11(2):88.
7. Tao N, Li K, Liu J. Molecular Mechanisms of Ferroptosis and Its Role in Pulmonary Disease. Oxid Med Cell Longev. 2020;2020(9547127.
8. Xie Y, Hou W, Song X, et al. Ferroptosis: process and function. Cell Death Differ. 2016;23(3):369–79.
9. Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell. 2017;171(2):273–285.
10. Hassannia B, Vandenabeele P, Vanden Berghe T. Targeting Ferroptosis to Iron Out Cancer. Cancer Cell. 2019;35(6):830–849.
11. Liang C, Zhang X, Yang M, Dong X. Recent Progress in Ferroptosis Inducers for Cancer Therapy. Adv Mater. 2019;31(51):e1904197.
12. Bersuker K, Hendricks JM, Li Z, et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature. 2019;575(7784):688–692.
13. Santana-Codina N, Gikandi A, Mancias JD. The Role of NCOA4-Mediated Ferritinophagy in Ferroptosis. Adv Exp Med Biol. 2021;1301(41–57.
14. Kroemer G, Mariño G, Levine B. Autophagy and the Integrated Stress Response. Molecular Cell. 2010;40(2):280–293.
15. Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. Nature Cell Biology. 2010;12(9):814–822.
16. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature. 2014;509(7498):105–109.
17. Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. Cell Res. 2016;26(9):1021–32.
18. Santana-Codina N, Mancias J. The Role of NCOA4-Mediated Ferritinophagy in Health and Disease. Pharmaceuticals. 2018;11(4):114.
19. Zheng B, Niu Z, Si S, et al. Comprehensive analysis of new prognostic signature based on ferroptosis-related genes in clear cell renal cell carcinoma. Aging (Albany NY). 2021;13(undefined):
20. Zhang A, Yang J, Ma C, Li F, Luo H. Development and Validation of a Robust Ferroptosis-Related Prognostic Signature in Lung Adenocarcinoma. Front Cell Dev Biol. 2021;9(616271.
21. Zhao Y, Zhao W, Lim YC, Liu T. Salinomycin-Loaded Gold Nanoparticles for Treating Cancer Stem Cells by Ferroptosis-Induced Cell Death. Mol Pharm. 2019;16(6):2532–2539.

22. Xu X, Zhang X, Wei C, et al. Targeting SLC7A11 specifically suppresses the progression of colorectal cancer stem cells via inducing ferroptosis. Eur J Pharm Sci. 2020;152(105450.
23. Deng F, Sharma I, Dai Y, Yang M, Kanwar YS. Myo-inositol oxygenase expression profile modulates pathogenic ferroptosis in the renal proximal tubule. J Clin Invest. 2019;129(11):5033–5049.
24. Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y. CD44 in cancer. Crit Rev Clin Lab Sci. 2002;39(6):527–79.
25. Leung EL, Fiscus RR, Tung JW, et al. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One. 2010;5(11):e14062.
26. Gilsanz A, Sánchez-Martín L, Gutiérrez-López MD, et al. ALCAM/CD166 adhesive function is regulated by the tetraspanin CD9. Cell Mol Life Sci. 2013;70(3):475–93.
27. Zhang WC, Shyh-Chang N, Yang H, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell. 2012;148(1–2):259–72.
28. Yan X, Luo H, Zhou X, Zhu B, Wang Y, Bian X. Identification of CD90 as a marker for lung cancer stem cells in A549 and H446 cell lines. Oncol Rep. 2013;30(6):2733–40.
29. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1–6.
30. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-w102.
31. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19(8):649–658.
32. Bartha Á, Győrffy B. TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. Int J Mol Sci. 2021;22(5):
33. Uhlén M, Björling E, Agaton C, et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics. 2005;4(12):1920–32.
34. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res. 2017;77(21):e108-e110.
35. Lánczky A, Nagy Á, Bottai G, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Res Treat. 2016;160(3):439–446.
36. Anaya J. OncoLnc: linking TCGA survival data to mRNAs, miRNAs, and lncRNAs. PeerJ Computer Science. 2016;2(e67.
37. Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 2010;38(Web Server issue):W214-20.
38. Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45(D1):D362-d368.

39. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46(D1):D956-d963.
40. Malta TM, Sokolov A, Gentles AJ, et al. Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation. Cell. 2018;173(2):338–354.e15.
41. Lian H, Han Y-P, Zhang Y-C, et al. Integrative analysis of gene expression and DNA methylation through one-class logistic regression machine learning identifies stemness features in medulloblastoma. Molecular Oncology. 2019;13(10):2227–2245.
42. Ozkan E, Bakar-Ates F. Ferroptosis: A Trusted Ally in Combating Drug Resistance in Cancer. Curr Med Chem. 2021;
43. Friedmann Angeli JP, Krysko DV, Conrad M. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nat Rev Cancer. 2019;19(7):405–414.
44. Doll S, Freitas FP, Shah R, et al. FSP1 is a glutathione-independent ferroptosis suppressor. Nature. 2019;575(7784):693–698.
45. Yu H, Han Z, Xu Z, An C, Xu L, Xin H. RNA sequencing uncovers the key long non-coding RNAs and potential molecular mechanism contributing to XAV939-mediated inhibition of non-small cell lung cancer. Oncol Lett. 2019;17(6):4994–5004.
46. Qin Y, Qiao Y, Wang D, Tang C, Yan G. Ferritinophagy and ferroptosis in cardiovascular disease: Mechanisms and potential applications. Biomedicine & Pharmacotherapy. 2021;141(111872.
47. Santana-Codina N, Mancias JD. The Role of NCOA4-Mediated Ferritinophagy in Health and Disease. Pharmaceuticals (Basel, Switzerland). 2018;11(4):114.
48. Li Y, Tao L, Cai W. Profiles of Immune Infiltration and Prognostic Immunoscore in Lung Adenocarcinoma. Biomed Res Int. 2020;2020(5858092.
49. Pérez-Ruiz E, Melero I, Kopecka J, Sarmento-Ribeiro AB, García-Aranda M, De Las Rivas J. Cancer immunotherapy resistance based on immune checkpoints inhibitors: Targets, biomarkers, and remedies. Drug Resist Updat. 2020;53(100718.
50. Ngiow SF, Young A. Re-education of the Tumor Microenvironment With Targeted Therapies and Immunotherapies. Front Immunol. 2020;11(1633.
51. Bohn T, Rapp S, Luther N, et al. Tumor immunoevasion via acidosis-dependent induction of regulatory tumor-associated macrophages. Nat Immunol. 2018;19(12):1319–1329.

Tables

Table1. Correlation Between the Expression of NCOA4 and Gene Markers of Immune Cells.

Description	Gene markers	LUAD			
		None		Purity	
		Cor	P Value	Cor	P Value
T cell	CD3D	0.055	2.16e-01	0.045	3.14e-01
	CD3E	0.114	**	0.123	**
	FOXP3	0.092	*	0.094	*
	CD80	0.29	***	0.304	5.51e-02
CD8+T cell	CD8A	0.145	***	0.151	***
	CD8B	0.095	*	0.086	5.74e-02
B cell	CD19	-0.039	3.78e-01	-0.052	2.45e-01
	CD20□KRT20□	-0.044	3.14e-01	-0.054	2.28e-01
	CD79A	-0.015	7.33e-01	-0.019	6.69e-01
Macrophage	CD11b(ITGAM)	0.262	***	0.281	***
	CD68	0.345	***	0.368	***
M1 macrophage	CXCL10	0.147	***	0.152	***
	INOS(NOS2)	0.158	***	0.161	***
	IRF5	0.098	*	0.095	*
	ARG1	0.093	*	0.086	5.63e-02
	COX2(PTGS2)	-0.017	6.93e-01	-0.008	8.60e-01
M2 macrophage	CCL18	0.236	***	0.248	***
	CD163	0.429	***	0.457	***
	MRC1	0.406	***	0.415	***
	MS4A4A	0.425	***	0.454	***
TAM	CCL2	0.185	***	0.192	***
	CD80	0.29	***	0.304	***
	CD86	0.355	***	0.384	***
	CCR5	0.309	***	0.338	***
Monocyte	CD14	0.23	***	0.247	***
	CD16(FCGR3B)	0.386	***	0.39	***

Natural killer cell	KIR2DL3	0.117	**	0.117	**
	KIR2DL4	-0.012	7.8e-01	-0.01	8.17e-01
	KIR3DL1	0.076	8.52e-02	0.073	1.05e-01
	KIR3DL2	0.075	8.78e-02	0.064	1.58e-01
	KIR3DL3	-0.015	7.33e-01	-0.02	6.62e-01
	XCL1	0.015	7.37e-01	0	9.93e-01
	CD7	-0.092	*	-0.103	*
Neutrophil	CD66b(CECAM8)	0.214	***	0.212	***
Dendritic cell	CD83	0.258	***	0.259	***
	CD141(THBD)	0.26	***	0.261	***
	CD11c(ITGAX)	0.119	**	0.131	**
Th1	STAT4	0.086	5e-02	0.09	*
	TNF	0.164	***	0.162	***
	IFNG	0.056	2.03e-01	0.057	2.05e-01
	TBX21	0.079	7.48e-02	0.083	6.61e-02
	IL12RB2	0.11	1.25e-02	0.105	2.03e-02
	WSX1(IL27RA)	0.05	2.61e-01	0.041	3.63e-01
Th2	GATA3	0.045	3.08e-01	0.043	3.39e-01
	STAT6	0.275	***	0.273	***
	STAT5A	0.258	***	0.275	***
	CCR3	0.127	**	0.133	**
Tfh	BCL6	0.056	2.05e-01	0.055	2.19e-01
	ICOS	0.21	***	0.229	***
	CXCR5	0.058	1.92e-01	0.054	2.34e-01
Th17	STAT3	0.407	***	0.415	***
	IL17A	0.036	4.12e-01	0.029	5.16e-01
T cell exhaustion	CTLA4	0.023	5.97e-01	0.015	7.41e-01
	LAG3	-0.076	8.42e-02	-0.083	6.55e-02
	HAVCR2	0.322	***	0.352	***

PD-1(PDCD1)	-0.022	6.18e-01	-0.032	4.80e-01

TAM: tumor-associated macrophage; Th: T helper cell; Tfh: follicular helper T cell; Cor: R value of Spearman's correlation; none: correlation without adjustment; purity: correlation adjusted by purity. ∗P < 0.01; ∗∗P < 0.001; ∗∗∗P < 0.0001.

Figures

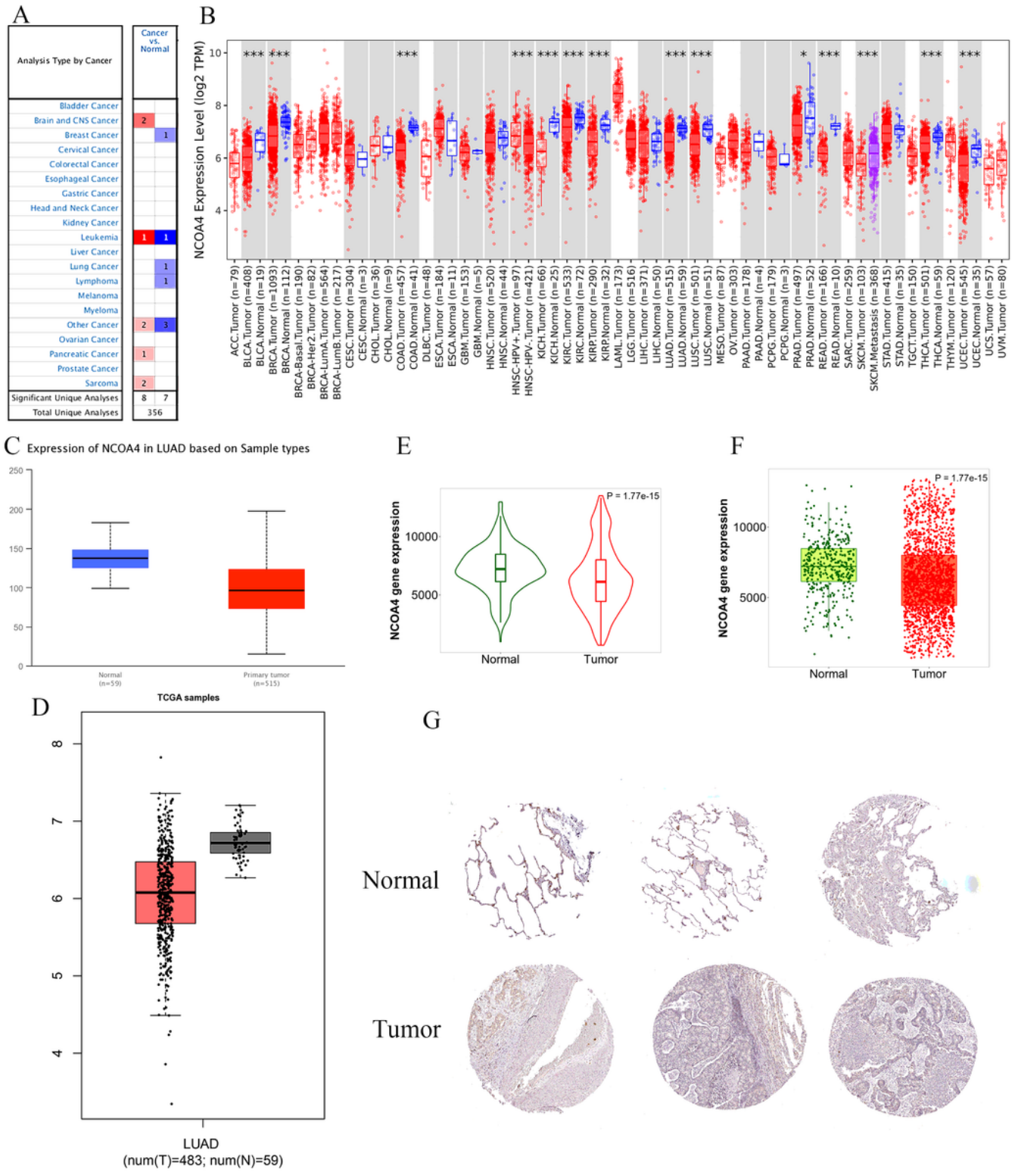

Figure 1

Expression of NCOA4 in lung adenocarcinoma. (A) Expression of NCOA4 in different types of tumor tissue and normal sample tissue in the Oncomine database. (B) Expression of NCOA4 in different human cancers in the TIMER database. (C)UALCAN database showed the expression level of NCOA4 in LUAD. (D) The expression of NCOA4 in LUAD was lower than normal tissue in GEPIA database. (E-F) TNM plot

database showed the expression of NCOA4 in LUAD. (G) Immunohistochemical staining of NCOA4 was showed in LUAD and normal tissues.

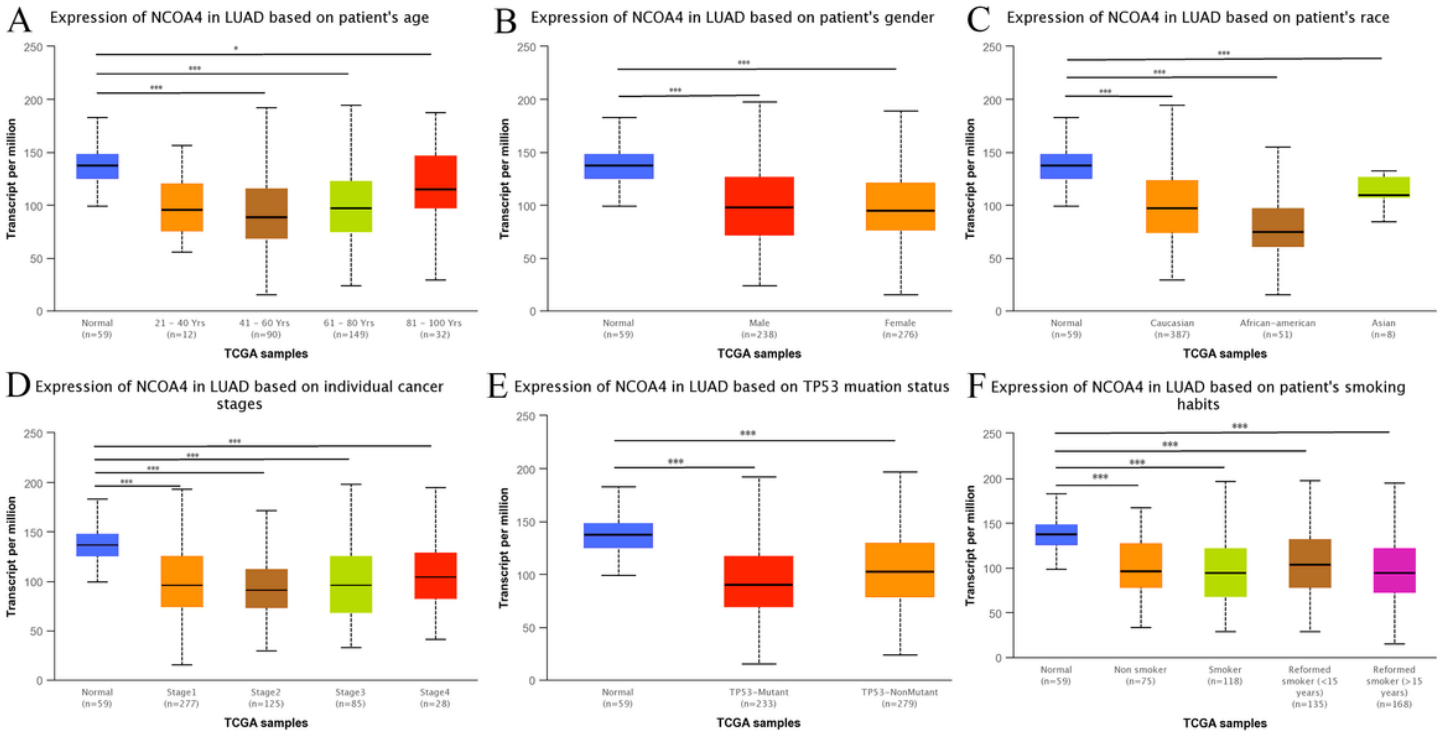

Figure 2

Correlation between NCOA4 expression and clinicopathological features. The relationship NCOA4 expression level was determined using the UALCAN database in (A)age, (B)gender, (C)race, (D)cancer stage, (E)TP53 muation status, (F)smoking habits.

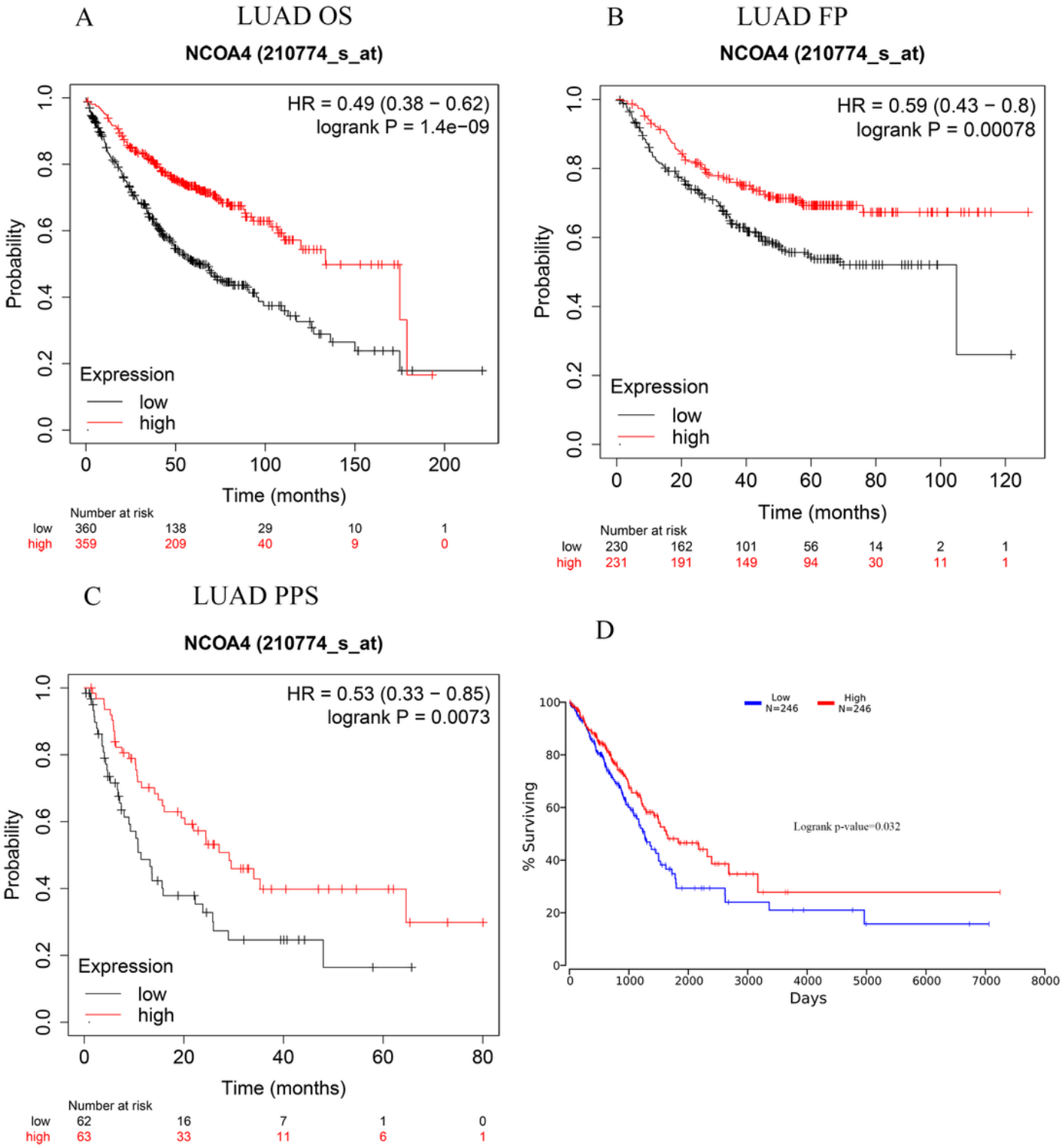

Figure 3

NCOA4 was associated with survival results. (A) The overall survival curve of NCOA4 for LUAD using the Kaplan-Meier plotter. (B) The FP curve of NCOA4 for LUAD using the Kaplan-Meier plotter. (C) The PPS curve of NCOA4 for LUAD using the Kaplan-Meier plotter. (D) Survival analysis of NCOA4 using OncoLnc database.

Figure 4

Correlation between NCOA4 expression and immune cell infiltration in LUAD. (A) NCOA4 expression was correlated with six major tumor-infiltrating immune cells in the TIMER database. (B) Red and blue represent tumor tissues and normal tissues, respectively. The upper left corner shows the method used for obtaining P-values and asterisks represent the levels of significance (G1: tumor; G2: normal). *P < 0.05, **P < 0.01, ***P < 0.001.

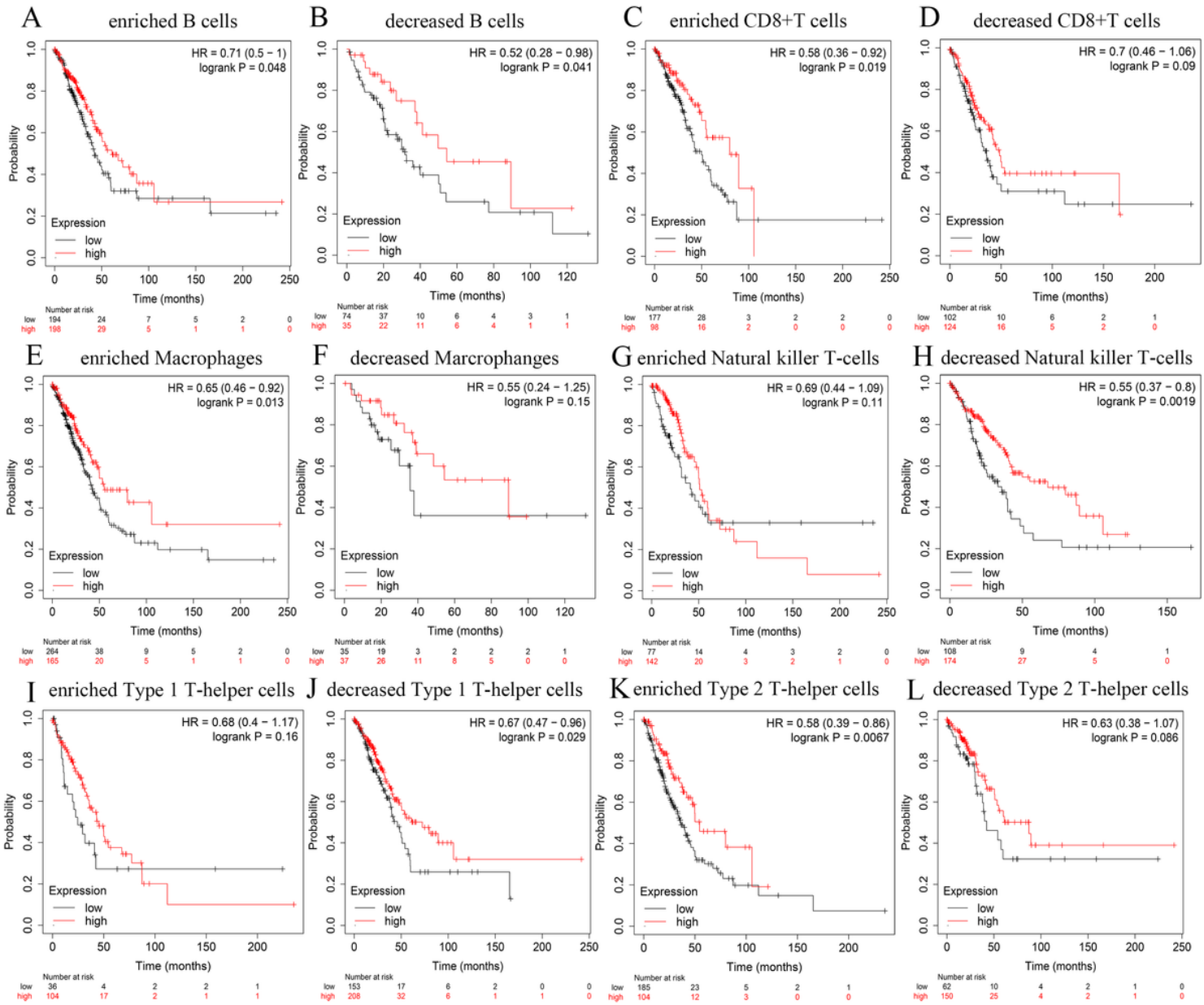

Figure 5

Effects of NCOA4 on survival based on different immune cell subgroups（A-F）.

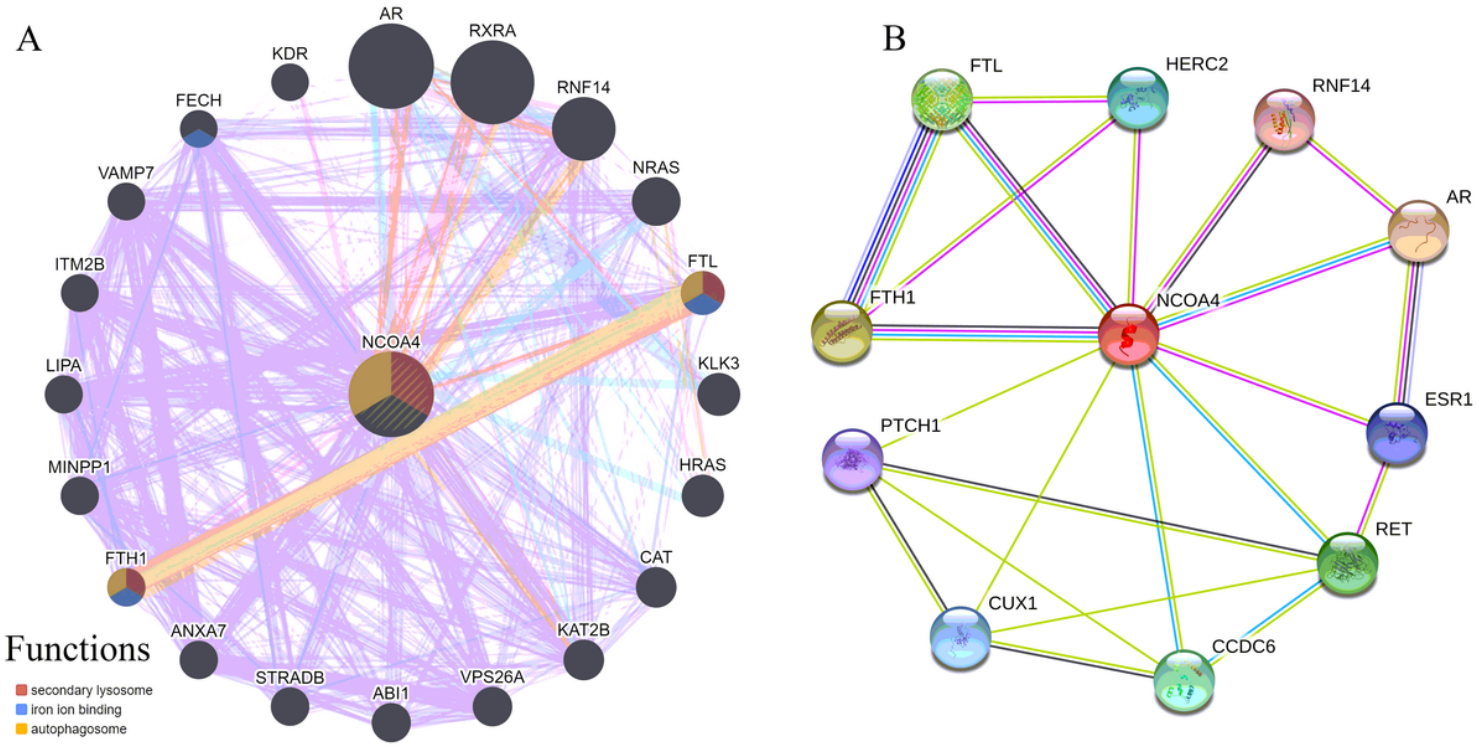

Figure 6

Interaction network of NCOA4. (A) An interactive network of NCOA4 was established through the GeneMANIA database. (B) A PPI network of NCOA4 was generated from STRING database.

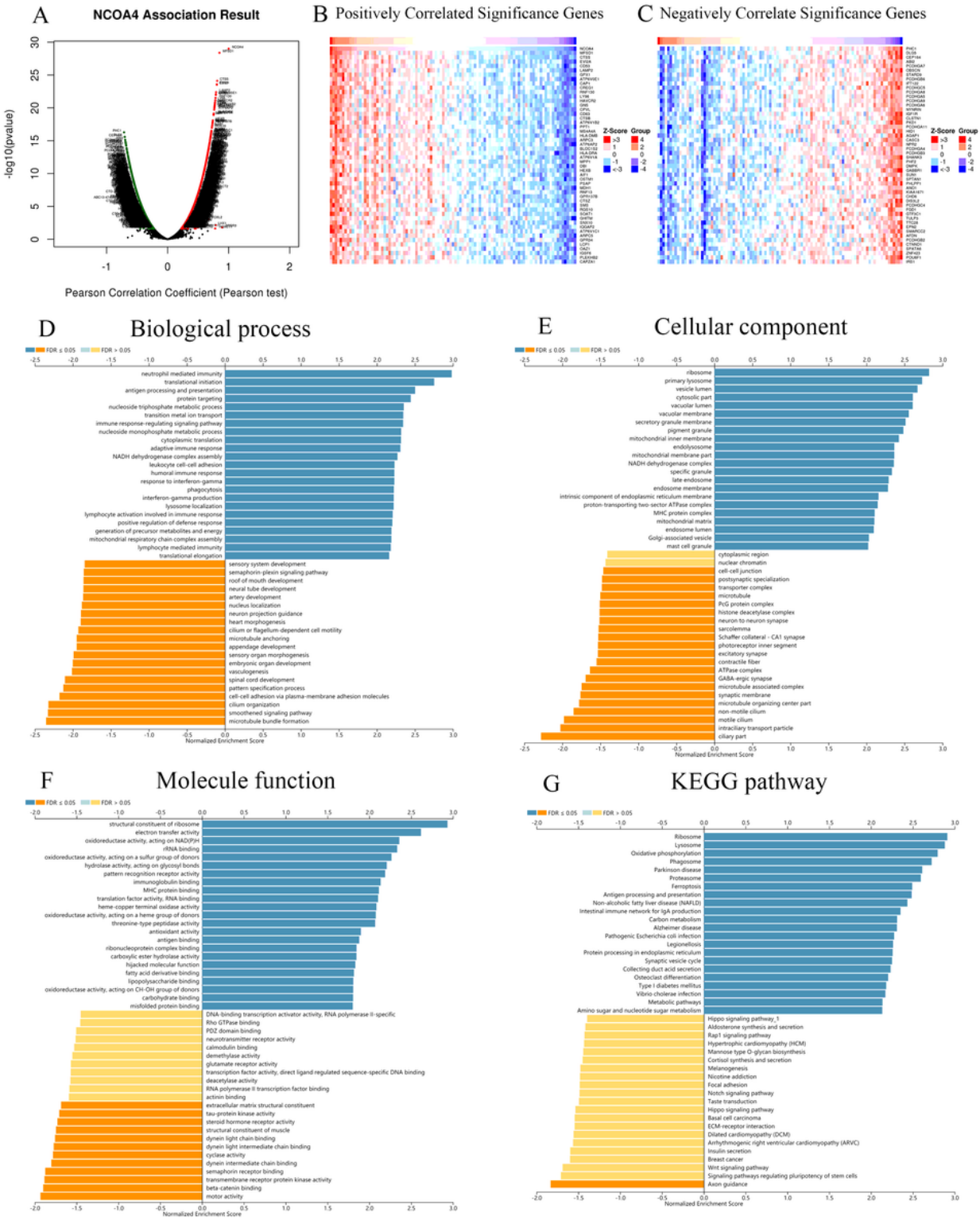

Figure 7

NCOA4 co-expression gene in LUAD. (A) The global NCOA4 highly correlated gene identified by the Pearson test in LUAD. Red ang green dots represent positively and negatively significantly correlated genes with NCOA4. (B, C) Heatmaps showing the top 50 gene positively and negatively correlation with NCOA4 in LUAD. (D-F) Enriched Gene Ontology annotations of NCOA4-correlated genes in LUAD.

Biological process (BP), Cell composition (CC), Molecular function (MF). (G) Enrichment pathway analysis of NCOA4-correlated genes in LUAD.

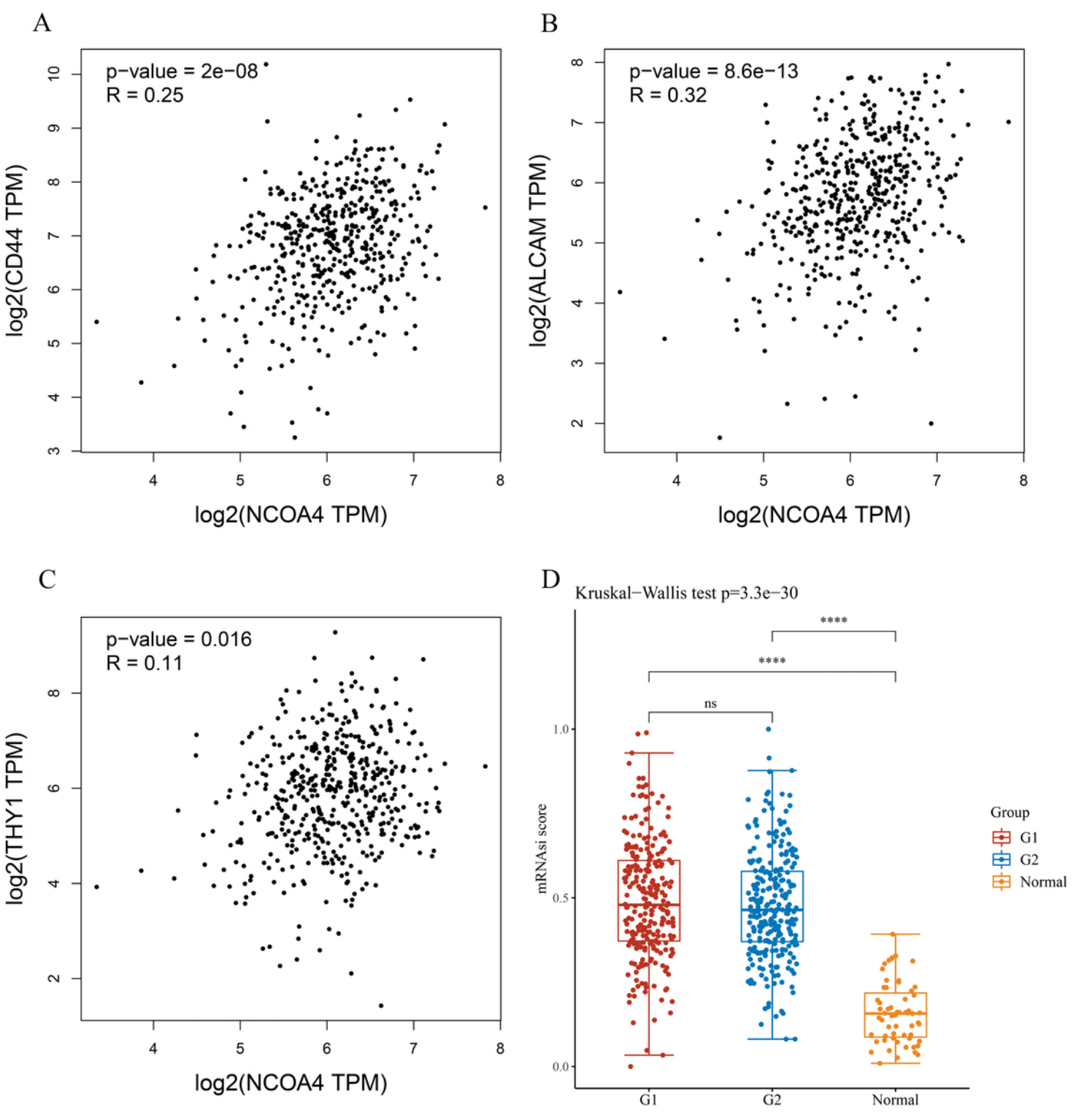

Figure 8

Correlation between NCOA4 and lung cancer stem cells. (A) NCOA4 is positively correlated with CD44. (B) NCOA4 is positively correlated with CD166. (C) NCOA4 is positively correlated with CD90. (D) mRNAsi

scores in different subgroups of lung adenocarcinoma and normal tissue.