Identification of the Expression Signature and Potential Mechanisms of miR-493-3p in NSCLC using Bioinformatics Strategy: A Comprehensive Study from TCGA and GEO Datasets

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Abstract

Background

Recent evidence highlights that miR-493-3p serve as crucial regulators of tumorigenesis. Nevertheless, the expression and clinical roles of miR-493-3p has been rarely reported in non-small cell lung cancer (NSCLC). Thus, this study was aim to investigate the expression status and potential mechanism of miR-493-3p in NSCLC progression.

Methods

We initially examined the expression of miR-493-3p in NSCLC through The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) microarrays. The overlap of the conjecture miR-493-3p target genes and down-regulated genes in NSCLC from TCGA were identified as the possible miR-493-3p target genes. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses and protein-protein interaction (PPI) network were constructed to explore the biological function and hub genes of miR-493-3p targets. The expression pattern and prognosis value of key hub genes were examined by Genotype-Tissue Expression (GTEx) database, The Human Protein Atlas and Kaplan- Meier Plotter database.

Results

miR-493-3p was significantly increased in NSCLC tissues and connected with tumor stage in TCGA and GEO database. A total of 46 genes were identified as miR-493-3p targets, and those involved in various key pathways by GO and KEGG analysis. Furthermore, PH domain and leucine-rich repeat protein phosphatase 2 (PHLPP2) was indicated of miR-493-3p key targets, which low-expressed in NSCLC and predicted better overall survival.

Conclusions

Our study emphasized that up-regulated miR-493-3p may target PHLLP2 and predicate worse prognosis of NSCLC patients.

Background

As one of the most morbidity and mortality malignant tumor, NSCLC account for 85% of the all diagnosed lung cancer, which can be further classified to lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) and large cell lung cancer (LCLC) according to the histological morphology[1,2]. Although, traditional treatment includes surgery, chemotherapy, radiotherapy and precise treatment such as target targeted therapy, immunotherapy made remarkable progress in treatments of NSCLC, and many biomarkers such as programmed death ligand 1 (PD-L1), tumor mutation burden (TMB) are regard as better prognostic or predictive biomarkers in NSCLC, patient's overall survival (OS) remains extremely low and unsatisfying[3-7]. Therefore, it is urgent to probe the initiating factors and mechanism of NSCLC and to boost the level of diagnosis and treatment for NSCLC.

miRNAs are a class of small non-coding RNA of approximately 20 nucleotides in size and can suppress the expression of multiple target genes through post-transcriptional regulation [8,9]. Recent studies have identified that miRNAs can act as tumor promoters or suppressors, and play pivotal roles in multiple malignant phenotype of tumors, including propagation, differentiation, chemotherapy-resistant, invasion and metastasis[10-14]. The different expression of miRNAs predict different prognosis in different caners[15,16], as well as NSCLC[17]. Despite these achievements, there is still much thought provoking mechanism urgent to explore between miRNAs and tumors.

miR-493-3p, which has been extensively studied in multiple cancers, such as breast cancer[18], liver cancer[19], osteosarcoma[20], pancreatic cancer[21], melanoma[22]. Most studies showed that the lower expression of miR-493-3p acted as a cancer suppressor and associated with poor prognosis[23,24]. However, other studies reported that up-regulated expression of miR-493-3p portend a poor prognosis[25]. Likewise, previous researches have showed silencing miR-493-3p expression increases the chemotherapy resistance in lung cancer[26]. However, the expression and specific role of miR-493-3p, as well as clinical parameters in NSCLC has not yet been elucidated.

Hence, this study was performed to ascertain the correlations between miR-493-3p expression and clinicopathologic parameters, the hub target genes of miR-493-3p and its clinical prognostic significance in NSCLC by series public database platform (Fig. 1). This study was aim to provide comprehensive information on the role of miR-493-3p in NSCLC progression.

Methods

Investigating the expression of miR-493-3p in TCGA database

miRNA gene sequencing of miR-493-3p in NSCLC and paracancerous tissues (PCT) were downloaded from TCGA database (https://cancergenome.nih.gov/). Then, we normalized the expression value of miR-493-3p by log2 transformation. The differences of miR-493-3p expression in the NSCLC tissues and the PCT were analyzed by SPSS Statistics V22.0. Kaplan-Meier methods were used to assess the overall survival (OS) of NSCLC patients with miR-493-3p high or low expression.

Analysis the expression status of miR-493-3p by GEO database

We screened and downloaded microarray data connected with miR-493-3p expression of NSCLC in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) deadline to September t 1th, 2019 with those search terms: (microRNA OR miRNA) AND Lung AND (cancer OR tumor). The inclusion criteria for research were as follows: (1) all cases were diagnosed NSCLC by pathological diagnosis; (2) each microarray has more than three NSCLC samples; (3) the expression information for miR-493-3p in NSCLC was reachable. All miR-493-3p expression values ware log2-transformed. Unpaired student's *t*-test was performed to analyze the expression status of miR-493-3p in NSCLC and paracancerous tissues.

Comprehensive meta-analysis the expression of miR-493-3p in GEO data

The expression mean (M) and standard deviation (SD) of miR-493-3p of eligible GEO microarrays were extracted to make comprehensive meta-analysis by Stata 12.0 software. Data in GEO microarrays were combined to obtain a value of standardized mean difference (SMD) and 95% confidential interval (CI) in forest plots. For the results of $\chi 2$ test, when $P < 0.01$ and $I^2 > 50\%$, GEO microarrays are significant heterogeneity, using the random

effects model to the pooling process. Sensitivity analyses were performed to assess the potential impact of microarrays in the heterogeneity. Then, publication bias was examined using funnel plots and the presence of asymmetry was assessed with Begg's and Egger's tests.

Bioinformaticsprediction of miR-493-3p targets

miRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/), an open online miRNA target genes prediction website that including 12 prediction databases. The target genes of miR-493-3p were screened by miRWalk must more than six prediction databases. Moreover, we extracted down-regulated genes in NSCLC by TCGA database through Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/). The overlapping genes, between miRWalk2.0 predicted target genes and down-regulated genes in TCGA database were identified promising target genes of the miR-493-3p, and were used in the further functional research.

GO and KEGG clustering analysis of miR-493-3p target genes

The database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/home.jsp), an online tool to perform the gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of miR-493-3p target genes in NSCLC. GO analysis is mainly composed by three parts as biological processes (BPs), cellular components (CCs) and molecular functions (MFs). The enrichment pathways were further optimizing by R package.

PPI network construction and hub target genes selection

The Search Tool for the Retrieval of Interacting Genes (STRING) (http://string-db.org/cgi/input.pl), which was used to construct a PPI network of miR-493-3p target genes. In addition, cytoHubba java plugin in Cytoscape v3.7.1 were employed to filter most likely hub genes in the PPI network.

Validating the expression and prognosis value of hub target genes

GEPIA was performed to describe the expression status of hub target genes of miR-493-3p in NSCLC and PCT, and the results present in box plots. Moreover, we investigated proteins expression status and clinical prognostic significance of hub target genes in NSCLC and PCT through The Human Protein Atlas (https://www.proteinatlas.org/) and the Kaplan Meier-plotter (https://kmplot.com/analysis/).

Results

Validation the clinicopathological value of miR-493-3p expression in NSCLC through TCGA database

In total, 500 samples of LUAD patients, 470 samples of LUSC patients, and 89 samples of PCT were collected from TCGA database. The miR-493-3p expression whether in LUAD, LUSC or in NSCLC, were remarkably up-regulated in tumor tissues compare with the normal controls (Fig. 2A-C). Moreover, we also performed Kaplan–Meier curve to investigate the influence of miR-493-3p expression on OS. No matter in LUAD, or in LUSC, there were no significant difference in OS between the patients with high miR-493-3p expression and the one with low levels with the *p*-values were all smaller than 0.05 (Fig. 2D-F). In addition, we also analyzed the correlation between miR-493-3p expression and clinicopathologic parameters of NSCLC, including age, gender, tumor stage, lymph node status and metastasis. As can be seen in Tables 1 and Tables 2, female patients (2.2581±1.2951)

had a higher expression level of miR-493-3p than male patients (2.0294±0.9327) in LUAD, and LUSC patients in stages I–II (2.5992±1.2113) had a higher expression level of miR-493-3p than those in stages III–IV (2.1763±0.9168). As for NSCLC patients, were composed by LUAD and LUSC patients, there was the significance in the statistics for the stages I–II (2.3717±1.1954) had a higher expression level of miR-493-3p than those in stages III–IV (2.1703±1.0768) (Table 3).

Confirmation the expression and meta-analysis of miR-493-3p in GEO database

After the selection criteria, we identified 12 eligible microarrays from the GEO database for further analysis, essential information and features of the included microarrays are depicted in Table 4. The expression of miR-493-3p for NSCLC in each of the included GEO database are illustrated in Fig. 3, the results indicated that the NSCLC patients had a prominently higher expression level of miR-493-3p than PCT in GSE27486, GSE15008 and GSE93300 (Fig. 3A-C), but no significance in other microarrays (Fig. 3D-L).To further confirm the expression of miR-493-3p in 12 included GEO database, a meta-analysis was performed. The heterogeneity test suggested prominent heterogeneity among included GEO database ($p < 0.05$, $I^2 = 70.2\%$). Hence, the random effects model was performed, as shown in Fig.4A, the forest plot of pooled SMD is 0.31 and 95% CIs is 0.07 to 0.54, which showed NSCLC tissues had higher miR-493-3p expression than PCT. Moreover, no GEO database led to a significant deviation from the overall pooled results through sensitivity analysis (Fig. 4B). Regarding publication bias, Bgger's test (p=0.150) and Egger's test (p=0.699) indicated there was no evidence of publication bias among all included GEO databases (Fig. 4C-D).

Collection the potential target genes of miR-493-3p by bioinformatics analyses

2103 potential targeted genes of miR-493-3p in NSCLC predicted by more than six prediction databases were obtained from miRWalk 2.0. In addition, a total of 1108 low-expressed genes in LUAD and 1920 low-expressed genes in LUSC were obtained from GEPIA. After intersection, 33 potential target genes of miR-493-3p were sorted out (Fig. 5A). Moreover, we screened literatures relevant to miR-493-3p targeted genes in the PubMed, and indentified 13 target genes of miR-493-3P including: AKT2, STK38L, HMGA2, E2F5, ETS1, FAM168A, PHLPP2, RSPO2, SP1, ANTXR1, STMN-1, KCNH2, ZFX [19-21,26-30]. Ultimately, a total of 46 potential target genes of miR-493-3p were identified for further analysis.

GO and KEGG enrichment analysis of potential target genes

To further explore the biological functions of miR-493-3p target genes, GO and KEGG analysis were performed by DAVID. For the GO analysis, 3 terms were gathered for biological processes (BPs) including hepatocyte proliferation (GO:0072574), epithelial tube branching involved in lung morphogenesis (GO:0060441), regulation of microtubule polymerization or depolymerization (GO:0031110), 3 terms were gathered for cellular components (CCs) including: cell surface (GO:0009986), transcription factor complex (GO:0005667), chromosome, centromeric region (GO:0000775), 3 terms were gathered for molecular functions (MFs) including: transcription factor activity and sequence-specific DNA binding (GO:0003700), transcription factor activity and RNA polymerase II core promoter proximal region sequence-specific binding (GO:0000982), DNA binding(GO:0003677) (Fig. 5B). For the KEGG analysis, 11 significant pathways were identified involving in NSCLC, cell cycle, PI3K-Akt signaling pathway, etc (Fig. 5C).These GO and KEGG enrichment pathways may be the key regulatory mechanisms of miR-493-3p in NSCLC progression.

Construction the PPI network and validation of hub genes

To further explore the hub genes of miR-493-3P targets, a PPI network comprises 45 nodes and 17 edges was constructed on STRING (Fig. 6), a single gene can connect with several genes, this intersect can form a functional complex involved in NSCLC progression. Moreover, we identified 9 hub genes of miR-493-3P (MKI67, MAD2L1, FEN1, SKA3, E2F1, SP1, ETS1, PHLPP2, TGFA) using MCODE module analysis in Cytoscape v3.7.1. The expression of 9 hub genes in NSCLC were analyzed in GEPIA, and found that two genes (PHLPP2, ETS1) lowly expressed in 486 LUSC patients and 483 LUAD patients compare to normal tissues(Fig. 7A-B), others found not dramatically different or higher expressed in NSCLC than normal tissues(Fig. 7C-I). Since miR-493-3p is overexpressed in NSCLC, the target genes of miR-493-3p are most likely low-expressed in NSCLC. However, ETS1 exhibits contradicted with the previous researches for highly expressed in NSCLC tissues compared with normal tissues [31-33]. Therefore, PHLPP2 was most likely to become target gene of miR-493-3p.

To assess the prognostic value of PHLPP2, we explore PHLPP2 protein expression in the Human Protein Atlas database, and found PHLPP2 was down-regulated in LUAD and LUSC compared to normal tissues (Fig.8A). Moreover, Kaplan-Meier curve analysis were performed to estimate the clinical prognostic significance of PHLPP2, the results indicated that high expression of PHLPP2 predict significantly longer survival (Fig. 8B).

Discussion

NSCLC, consisted of LUAD and LUSC chiefly, remains the leading reason of cancer mortality worldwide. Until the last decade, the 5-year overall survival rate for patients with metastatic NSCLC was less than 5%[34]. Further understanding of the biology molecular mechanism and finding new biomarks of NSCLC may provide novel therapeutic targets. Although previous study have documented miR-493-3p involved in the progression of NSCLC[35], the expression and molecular mechanism of miR-493-3p in NSCLC progression still not been clarified. In this study, we intended using a comprehensive bioinformatics analysis to investigate the expression, targets genes and potential molecular mechanism of miR-493-3p in NSCLC progression.

As one of the noncoding RNAs, microRNAs (miRNAs) are novel gene regulators that target the 3'-UTR of downstream mRNAs to accelerate their degradation and/or block their translations via seed region matching. Current studies have reported that miR-493-3p differentially expressed in multiple cancers, and had been defined as tumor suppressor which inhibits the progressions of several types of cancers. For example, miR-493-3p had been found down-regulated in leukemia cells and could affect leukemogenesis, clonogenic and stemness capacities[36]. Wang et al. revealed that miR-493-3p downregulated in laryngeal squamous cell carcinoma(LSCC), and LINC01605 directly target at miR-493-3p to promote LSCC proliferation[37]. Xu et al. found that miR-493-3p was downregulated in prostate cancer cells and regulated the expression of YTHDF2, which indirectly regulated N6-methyladenosine modification to inhibit the proliferation and migration abilities of prostate cancer cells[38]. However, the expression pattern and specific molecular mechanisms of miR-493-3p in NSCLC were still unclear.

In present study, up-regulated expression of miR-493-3p was identified in NSCLC by TCGA and GEO database. This results hints miR-493-3p may act as a tumor promoter in NSCLC progression. Although the Kaplan-Meier curves showed no significance difference between the low-expression miR-493-3p patients and the high-expression miR-493-3p patients from TCGA database, the clinical correlation analysis revealed that female patients had a higher expression of miR-493-3p than male patients in LUAD, and high expression of miR-493-3p was related to the tumor stages in LUSC patients. Moreover, the meta-analysis indicated NSCLC patients had

higher miR-493-3p expression than non-tumor patients in GEO database. Due to that miR-493-3p function with target genes, bioinformatics analyses were performed to discover the potential target genes and specific molecular mechanisms of miR-493-3p in NSCLC. Based on miRWalk2.0 and GEPIA, the intersection including 33 potential target genes were selected, and 13 target genes of miR-493-3P through literature screening and extraction. Finally, a total of 46 candidate target genes were identified.

To confirm the functions of 46 target genes, GO and KEGG enrichment analyses were performed. As for the GO enrichment analysis, we found that hepatocyte proliferation, epithelial tube branching involved in lung morphogenesis and regulation of microtubule polymerization or depolymerization were the main functions for BPs, and the functions including cell surface, transcription factor complex, chromosome, centromeric region were the main terms for CCs, 3 terms were the main functions for MFs including transcription factor activity and sequence-specific DNA binding, transcription factor activity and RNA polymerase II core promoter proximal region sequence-specific binding, DNA binding. For the KEGG analysis, we identified 11 significant pathways that might play vital functions in NSCLC progression, including PI3K-Akt signaling pathway, cell cycle. This results consistent with the previous research about miR-493-3p functions in tumor progression, xu et al. revealed that miR-493-3p acts as a negative regulator in hepatocellular carcinoma progression which suppressed the expression of ANTXR1 and RSPO2[39]. Yu et al. also revealed that miR-493-3p can inactive the PI3K-Akt pathway to inhibited proliferation and metastasis of osteosarcoma cells[40]. Those significant GO and KEGG enrichment pathways indicated that those target genes of miR-493-3p may pay important role in NSCLC progression.

Basis on the PPI network, nine hub genes (MKI67, MAD2L1, FEN1, SKA3, E2F1, SP1, ETS1, PHLPP2, TGFA) were identified. Since miR-493-3p is up-regulated in NSCLC, the target genes of miR-493-3p have greater potential low-expressed in NSCLC, so we found ETS1 and PHLPP2 were lowly expressed in NSCLC patients compare with the normal tissues through GEPIA database, but EST1 was opposite to the previous studies for high expression in NSCLC tissues[41-43], PHLPP2 lowly expressed in NSCLC tissues and consistent with the previous studies[44,45] , and we also fund PHLPP2 lowly expressed in LUAD and LUSC tissues compare to normal lung tissues through Human Protein Atlas database. Moreover, Kaplan-Meier curve analysis indicated that NSCLC patients with high expression of PHLPP2 had a better OS. Therefore, PHLPP2 may be the most likely target gene of miR-493-3p. PHLPP2 catalyzes the dephosphorylation of Akt kinase, reduces the activity and expression level of Akt to suppress tumor growth[46]. Previous studies have showed that PHLPP2 expression is ubiquitously lost in multiple cancers and plays a key role in a wide range of biological behavior, such as cancer cell propagation, metastasis, autophagy and apoptosis[47-49]. As for NSCLC, wang et al, found PHLPP2 expression less pronounced in NSCLC tissue samples than that in non-tumor lung tissues and associated with the presence of lymph node metastasis[44]. Mei et al, found that the up-regulated miR-141 can direct target and suppress PHLPP2 to promote the proliferation of NSCLC[50]. Therefore, miR-493-3p might target at PHLPP2 to play an important role in NSCLC progression.

Conclusion

This research demonstrated that miR-493-3p was highly expressed in NSCLC and might target at PHLPP2 to promote NSCLC progression based on online, comprehensive, large database. Certainly, much effort and research is still needed to verify the functions of miR-493-3p in NSCLC progression, which is a challenging but promising task. Those findings suggest miR-493-3p might function as a latent tumor biomarker in the prognosis prediction for NSCLC, and pave the way for clinical NSCLC treatment and future molecular mechanism exploration.

Abbreviations

NSCLC: Non-small cell lung cancer; TCGA : The Cancer Genome Atlas; GEO: Gene Expression Omnibus; GO: Gene Ontology ; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: Protein-protein interaction; GTEx: Genotype-Tissue Expression; PHLPP2: PH domain and leucine-rich repeat protein phosphatase 2; LUSC: Lung squamous cell carcinoma; LUAD: Lung adenocarcinoma; LCLC:Large cell lung cancer; PD-L1: Programmed death ligand 1; TMB: Tumor mutation burden; OS: Overall survival; PCT: Paracancerous tissues; M: Mean; SD: Standard deviation; SMD: Standardized mean difference; CI: Confidential interval; GEPIA: Gene Expression Profiling Interactive Analysis; DAVID: The database for Annotation, Visualization and Integrated Discovery; BPs: Biological processes; CCs:Cellular components; MFs: Molecular functions; STRING: The Search Tool for the Retrieval of Interacting Genes.

Declarations

Ethics approval and consent to participate

Not applicable. All data in this study are publicly available.

Consent for publication

Not applicable.

Availability of data and materials

All analyzed data are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZH performed databases collection and analysis, and drafted the initial manuscript. ZHB and LM made substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data. In addition XQC was involved in drafting the manuscript and revising it critically for important intellectual content. ZLJ acquired the data and performed the analysis and interpreted the data. All authors read and approved the final manuscript.

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References

1. Rebecca LS, Kimberly DM, Ahmedin J. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7-34.
2. Kentaro I. Lung Cancer: Understanding Its Molecular Pathology and the 2015 WHO Classification. Front Oncol. 2017;7:193.
3. Noronha V, Patil VM, Joshi A, Menon N, Chougule A, Mahajan A, et al. Gefitinib Versus Gefitinib Plus Pemetrexed and Carboplatin Chemotherapy in -Mutated Lung Cancer. J. Clin Oncol, 38:124-136, 2019
4. Baker S, Dahele M, Lagerwaard FJ, Senan S. A critical review of recent developments in radiotherapy for non-small cell lung cancer. Radiat Oncol. 2016;11:115.
5. Ricciuti B, Dahlberg SE, Adeni A, Sholl LM, Nishino M, Awad MM. Immune Checkpoint Inhibitor Outcomes for Patients With Non-Small-Cell Lung Cancer Receiving Baseline Corticosteroids for Palliative Versus Nonpalliative Indications. J. Clin. Oncol, 2019;37: 1927-1934
6. Devarakonda S, Rotolo F, Tsao MS, Lanc I, Brambilla E, Masood A, et al. Tumor Mutation Burden as a Biomarker in Resected Non-Small-Cell Lung Cancer. J. Clin Oncol, 2018; 36: 2995-3006
7. Del C Monroig-Bosque P, Driver B, Morales-Rosado JA, Deavers M, Tacha D, Bernicker E, et al. Correlation Between Programmed Death Receptor-1 Expression in Tumor-Infiltrating Lymphocytes and Programmed Death Ligand-1 Expression in Non-Small Cell Lung Carcinoma. Arch Pathol Lab Med. 2018;142:1388-1393.
8. Mori MA, Ludwig RG, Garcia-Martin R, Brandão BB, Kahn CR. Extracellular miRNAs: From Biomarkers to Mediators of Physiology and Disease. Cell metabolism. 2019; 30: 656-673
9. Grigelioniene G, Suzuki HI, Taylan F, Mirzamohammadi F, Borochowitz ZU, Ayturk UM, et al. Gain-of-function mutation of microRNA-140 in human skeletal dysplasia. Nat Med. 2019;25:583-590.
10. Liu Q, Guan Y, Li Z, Wang Y, Liu Y, Cui R, et al. miR-504 suppresses mesenchymal phenotype of glioblastoma by directly targeting the FZD7-mediated Wnt-β-catenin pathway. J. Exp. Clin. Cancer Res, 2019; 38: 358
11. Ji Y, Fioravanti J, Zhu W, Wang H, Wu T, Hu J, et al. miR-155 harnesses Phf19 to potentiate cancer immunotherapy through epigenetic reprogramming of CD8 T cell fate. Nat Comm, 2019;10(1):2157
12. van Jaarsveld MT, van Kuijk PF, Boersma AW, Helleman J, van IJcken WF, Mathijssen RH, et al. miR-634 restores drug sensitivity in resistant ovarian cancer cells by targeting the Ras-MAPK pathway. Mol Cancer. 2015;14:196.
13. Valeri N, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. Cancer Cell. 2014;25:469-483.
14. Yang D, Sun Y, Hu L, Zheng H, Ji P, Pecot CV, et al. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell. 2013;23:186-199.
15. Kumarasamy C, Madhav MR, Sabarimurugan S, Krishnan S, Baxi S, Gupta A, et al. Prognostic Value of miRNAs in Head and Neck Cancers: A Comprehensive Systematic and Meta-Analysis. Cells, 2019;8,25
16. Ravegnini G, Cargnin S, Sammarini G, Zanotti F, Bermejo JL, Hrelia P, et al. Prognostic Role of miR-221 and miR-222 Expression in Cancer Patients: A Systematic Review and Meta-Analysis. Cancers (Basel). 2019;11.
17. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, et al. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. Cancer Cell. 2010;18:282-293.

18. Yao L, Liu Y, Cao Z, Li J, Huang Y, Hu X, et al. MicroRNA-493 is a prognostic factor in triple-negative breast cancer. Cancer Sci. 2018;109:2294-2301.
19. Ding W, Tan H, Li X, Zhang Y, Fang F, Tian Y, et al. MicroRNA-493 suppresses cell proliferation and invasion by targeting ZFX in human hepatocellular carcinoma. Cancer biomarkers. 2018;22:427-434.
20. Qian M, Gong H, Yang X, Zhao J, Yan W, Lou Y, et al. MicroRNA-493 inhibits the proliferation and invasion of osteosarcoma cells through directly targeting specificity protein 1. Oncol Lett. 2018;15:8149-8156.
21. Zhi D, Zhao X, Dong M, Yan C. miR-493 inhibits proliferation and invasion in pancreatic cancer cells and inversely regulated hERG1 expression. Oncol Lett. 2017;14:7398-7404.
22. Cui A, Jin Z, Gao Z, Jin M, Zhu L, Li L, et al. Downregulation of miR-493 promoted melanoma proliferation by suppressing IRS4 expression. Tmour Biol, 2017;39(5):1010428317701640.
23. Zhao J, Xu T, Wang F, Cai W, Chen L. miR-493-5p suppresses hepatocellular carcinoma cell proliferation through targeting GP73. Biomed. Pharmacother,2017; 90: 744-751
24. Ueno K, Hirata H, Majid S, Yamamura S, Shahryari V, Tabatabai ZL, et al. Tumor suppressor microRNA-493 decreases cell motility and migration ability in human bladder cancer cells by downregulating RhoC and FZD4. Mol Cancer Ther. 2012;11:244-253.
25. Jia X, Li N, Peng C, Deng Y, Wang J, Deng M, et al. miR-493 mediated DKK1 down-regulation confers proliferation, invasion and chemo-resistance in gastric cancer cells. Oncotarget. 2016;7:7044-7054.
26. Gu Y, Zhang Z, Yin J, Ye J, Song Y, Liu H, et al. Epigenetic silencing of miR-493 increases the resistance to cisplatin in lung cancer by targeting tongue cancer resistance-related protein 1(TCRP1). J. Exp. Clin. Cancer Res ,2017;36: 114
27. Kleemann M, Schneider H, Unger K, Bereuther J, Fischer S, Sander P, et al. Induction of apoptosis in ovarian cancer cells by miR-493-3p directly targeting AKT2, STK38L, HMGA2, ETS1 and E2F5. Cell. Mol. Life Sci, 2019;76: 539-559
28. Deng J, Ma M, Jiang W, Zhang H, Cui S. miR-493 Promotes Prostate Cancer Cells Proliferation by Targeting PHLPP2 and Activating Akt Signaling Pathway. Clinical laboratory. 2019;65(3): doi:10.7754/Clin.Lab.2018.180806.
29. Liu T, Liu L, Liu M, Du R, Dang Y, Bai M, et al. MicroRNA-493 targets STMN-1 and promotes hypoxia-induced epithelial cell cycle arrest in G/M and renal fibrosis. FASEB journa, 2019;33(2):1565-1577
30. Xu Y, Ge K, Lu J, Huang J, Wei W, Huang Q. MicroRNA-493 suppresses hepatocellular carcinoma tumorigenesis through down-regulation of anthrax toxin receptor 1 (ANTXR1) and R-Spondin 2 (RSPO2). Biomedicine & pharmacotherapy. 2017;93:334-343.
31. Kaur J, Tikoo K. Ets1 identified as a novel molecular target of RNA aptamer selected against metastatic cells for targeted delivery of nano-formulation. Oncogene. 2015;34:5216-5228.
32. Cao B, Tan S, Tang H, Chen Y, Shu P. miR-512-5p suppresses proliferation, migration and nvasion, and induces apoptosis in non-small cell lung cancer cells by targeting ETS1. Mol Med Report. 2019;19:3604-3614.
33. Yang YB, Tan H, Wang Q. MiRNA-300 suppresses proliferation, migration and invasion of non-small cell lung cancer via targeting ETS1. Eur Rev Med Pharmacol Sci. 2019;23:10827-10834.
34. R. S. Herbst, D. Morgensztern, C. Boshoff. The biology and management of non-small cell lung cancer. Nature. 2018;553:446-454.

35. Gu Y, Cheng Y, Song Y, Zhang Z, Deng M, Wang C, et al. MicroRNA-493 suppresses tumor growth, invasion and metastasis of lung cancer by regulating E2F1. PLoS One. 2014;9:e102602.
36. Valiollahi E, Ribera JM, Genescà E, Behravan J. Genome-wide identification of microRNA signatures associated with stem/progenitor cells in Philadelphia chromosome-positive acute lymphoblastic leukemia. Mol Biol Rep. 2019;46:1295-1306.
37. Wang XY, Wang L, Xu PC, Huang FJ, Jian X, Wei ZC, et al. LINC01605 promotes the proliferation of laryngeal squamous cell carcinoma through targeting miR-493-3p. Eur Rev Med Pharmacol Sci. 2019;23:10379-10386.
38. Li J, Meng S, Xu M, Wang S, He L, Xu X, et al. Downregulation of N-methyladenosine binding YTHDF2 protein mediated by miR-493-3p suppresses prostate cancer by elevating N-methyladenosine levels. Oncotarget. 2018;9:3752-3764.
39. Xu Y, Ge K, Lu J, Huang J, Wei W, Huang Q. MicroRNA-493 suppresses hepatocellular carcinoma tumorigenesis through down-regulation of anthrax toxin receptor 1 (ANTXR1) and R-Spondin 2 (RSPO2). Biomedicine & pharmacotherapy. 2017;93:334-343.
40. Zhang Z, Luo G, Yu C, Yu G, Jiang R, Shi X. MicroRNA-493-5p inhibits proliferation and metastasis of osteosarcoma cells by targeting Kruppel-like factor 5. J Cell Physiol. 2019;234:13525-13533.
41. Millien G, Cao Y, O'Hara CJ, Tagne JB, Hinds A, Williams MC, et al. ETS1 regulates Twist1 transcription in a Kras/Lkb1 metastatic lung tumor model of non-small cell lung cancer. Clin Exp Metastasis. 2018;35:149-165.
42. Zhou X, Zhou R, Zhou H, Li Q, Hong J, Meng R, et al. ETS-1 Induces Endothelial-Like Differentiation and Promotes Metastasis in Non-Small Cell Lung Cancer. Cell. Physiol. Biochem, 2018;45:1827-1839.
43. Taylor MA, Wappett M, Delpuech O, Brown H, Chresta CM. Enhanced MAPK signaling epigenetic modifications in a subset of lung SCC. Oncogene. 2016;35:4345-4357.
44. Wang H, Gu R, Tian F, Liu Y, Fan W, Xue G, et al. PHLPP2 as a novel metastatic and prognostic biomarker in non-small cell lung cancer patients. Thoracic cancer. 2019;10:2124-2132.
45. Huang H, Pan X, Jin H, Li Y, Zhang L, Yang C, et al. PHLPP2 Downregulation Contributes to Lung Carcinogenesis Following B[a]P/B[a]PDE Exposure. Clin. Cancer Res, 2015;21:3783-3793.
46. Toivanen R, Furic L. A balancing act: PHLPP2 fine tunes AKT activity and MYC stability in prostate cancer. J. Cell Biol, 2019;218:1771-1772
47. Wu SH, Xu XY, Sun CB, Wen FF, He S, Gao XQ, et al. Expression of PHLPP2 correlates with clinicopathologic characteristics and prognosis in colorectal cancer. Int J Clin Exp Pathol. 2019;12:2909-2919.
48. Chen S, Zhang JY, Sun LS, Li XF, Bai JY, Zhang HY, et al. miR-762 Promotes Malignant Development of Head and Neck Squamous Cell Carcinoma by Targeting PHLPP2 and FOXO4. Onco Targets Ther. 2019;12:11425-11436.
49. Nowak DG, Katsenelson KC, Watrud KE, Chen M, Mathew G, D'Andrea VD, et al. The PHLPP2 phosphatase is a druggable driver of prostate cancer progression. J. Cell Biol,2019; 218: 1943-1957.
50. Mei Z, He Y, Feng J, Shi J, Du Y, Qian L, et al. MicroRNA-141 promotes the proliferation of non-small cell lung cancer cells by regulating expression of PHLPP1 and PHLPP2. FEBS Lett. 2014;588:3055-3061.

Tables

Table 1 Association between miR-493-3p expression and clinicopathological parameters in LUAD patients based on TCGA data.

Clinicopathological		n	mean±SD	*p*-value
Tissue	Adjacent normal tissue	44	1.2560±0.5629	<0.0001
	LUAD	512	2.1510±1.1456	
Age	<60	135	2.1694±0.8387	0.841
	≥60	365	2.1462±1.2426	
Gender	Female	269	2.2581±1.2951	0.026
	Male	231	2.0294±0.9327	
Tumor stage	Stage □-□	393	2.1489±1.1363	0.8939
	Stage □-□	107	2.1656±1.1887	
T	T1-T2	433	2.1873±1.1819	0.0846
	T3-T4	67	1.9276±0.8623	
N	No	322	2.1844±1.1885	0.4028
	Yes	178	2.0947±1.0676	
M	No	341	2.1330±1.1122	0.5788
	Yes	159	2.1942±1.2194	

T, tumor; N, lymph node; M, metastasis; SD, standard deviation

Table 2 Association between miR-493-3p expression and clinicopathological parameters in LUSC patients based on TCGA data.

Clinicopathological		n	mean±SD	*p*-value
Tissue	Adjacent normal tissue	45	1.9233±0.6484	<0.0001
	LUSC	471	2.5227±1.1749	
Age	<60	85	2.6451±1.2068	0.2886
	≥60	385	2.4957±1.1660	
Gender	Female	122	2.3964±1.1113	0.1674
	Male	348	2.5670±1.1932	
Tumor stage	Stage Ⅰ-Ⅳ	385	2.5992±1.2113	0.0026
	Stage Ⅰ-Ⅱ	85	2.1763±0.9168	
T	T1-T2	381	2.5612±1.1944	0.1416
	T3-T4	89	2.3580±1.0719	
N	No	297	2.5885±1.2215	0.1113
	Yes	173	2.4097±1.0809	
M	No	386	2.4724±1.2144	0.0464
	Yes	84	2.7538±0.9391	

T, tumor; N, lymph node; M, metastasis; SD, standard deviation

Table 3 Association between miR-493-3p expression and clinicopathological parameters in NSCLC patients based on TCGA data.

Clinicopathological		n	mean±SD	*p*-value
Tissue	Adjacent normal tissue	89	1.5934±0.6932	<0.0001
	NSCLC	982	2.3299±1.1745	
Age	<60	220	2.3532±1.0237	0.7595
	≥60	750	2.3256±1.2165	
Gender	Female	391	2.3013±1.2408	0.5057
	Male	579	2.3525±1.1279	
Tumor stage	Stage □-□	778	2.3717±1.1954	0.0334
	Stage □-□	192	2.1703±1.0768	
T	T1-T2	814	2.3622±1.2023	0.0655
	T3-T4	156	2.1731±1.0101	
N	No	619	2.3786±1.2212	0.101
	Yes	351	2.2499±1.0842	
M	No	727	2.3132±1.1798	0.3931
	Yes	243	2.3876±1.1613	

T, tumor; N, lymph node; M, metastasis; SD, standard deviation

Table 4 Features of the enrolled GEO datasets in current study.

Database	Platform	Year	Type	NSCLC			Normal			*P*-value
				n	M	SD	n	M	SD	
GSE16025	GPL5106	2013	Tissue	61	5.5579	0.2822	10	5.5354	0.2223	0.814
GSE15008	GPL8176	2012	Tissue	187	7.139	1.3113	188	6.3309	1.1693	<0.001
GSE61741	GPL9040	2017	Blood	58	4.9729	1.0887	79	4.8683	1.4451	0.646
GSE46729	GPL8786	2014	Blood	24	3.9499	0.1683	24	4.0158	0.248	0.297
GSE14936	GPL8879	2012	Tissue	18	7.6915	0.5865	17	7.4566	0.4502	0.208
GSE25508	GPL7731	2014	Tissue	24	6.1212	0.1668	34	6.0486	0.2493	0.227
GSE27486	GPL11432	2012	Blood	22	0.7677	0.1928	13	0.6259	0.1592	0.037
GSE36681	GPL8179	2019	Tissue	103	9.4005	0.6475	103	9.3104	0.9049	0.414
GSE40738	GPL16016	2019	Blood	82	0.5279	0.1139	59	0.5029	0.1229	0.153
GSE63805	GPL18410	2016	Tissue	32	4.8243	0.7155	31	4.6832	0.7668	0.4637
GSE74190	GPL19622	2015	Tissue	66	0.2172	0.0483	44	0.1994	0.0481	0.802
GSE93300	GPL21576	2017	Blood	9	-6.044	0.3318	4	-8.187	0.5109	0.004

M, mean; SD, standard deviation

Figures

FIG.1

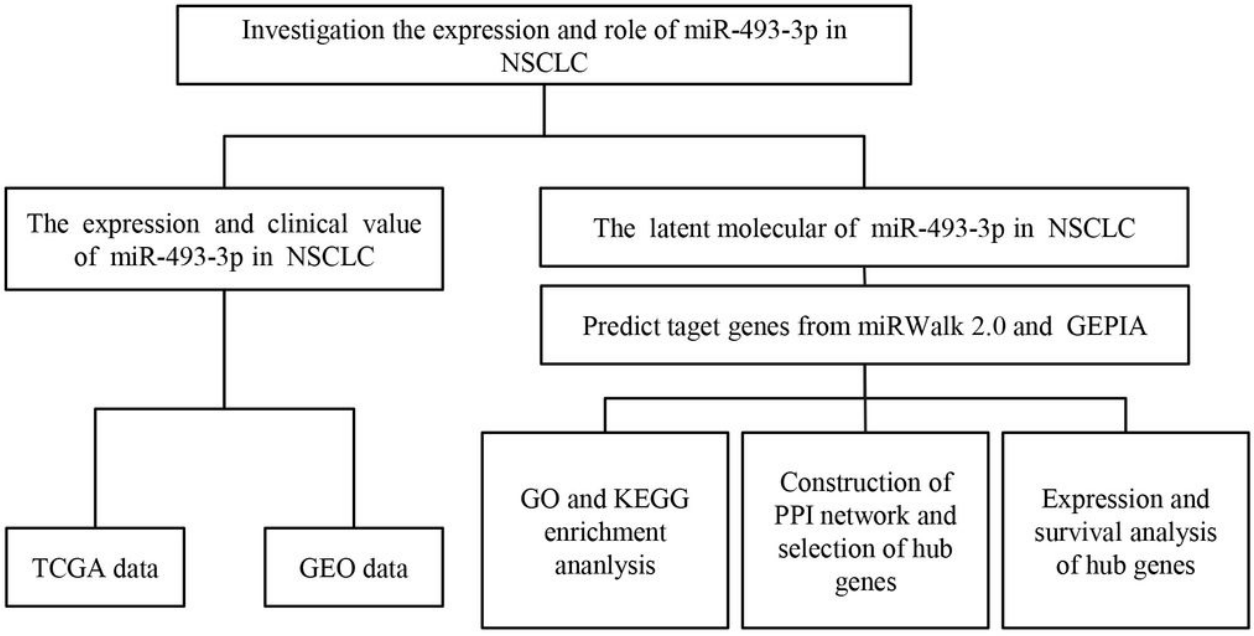

Figure 1

Overall flow chart. The current study were comprehensively analyzed based on multiple online database: validation miR-493-3p expression in NSCLC by TCGA and GEO databases, collection of the possible miR-493-3p target genes and potent molecular mechanism through GEPIA, MiRWalk2.0, DAVID, STRING, and GEPIA databases.

FIG.2

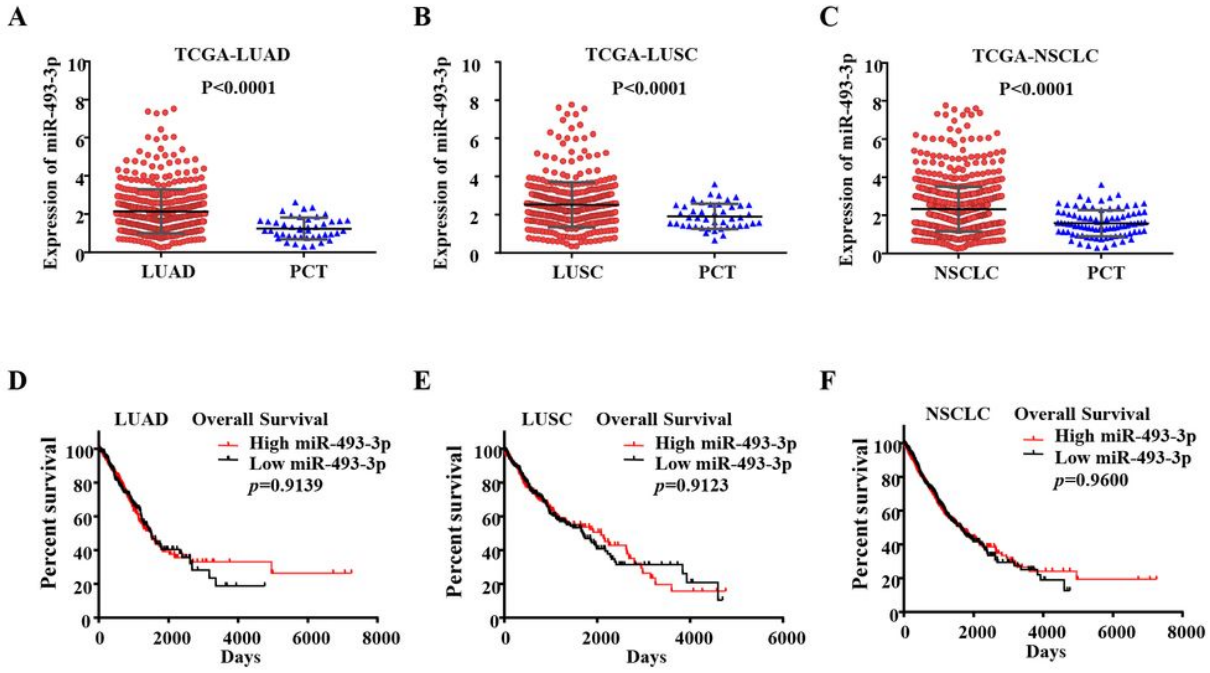

Figure 2

Expression and Kaplan-Meier survival curves of miR-493-3p in NSCLC based on TCGA data. A. miR-493-3p expression in LUAD and PCT. B. miR-493-3p expression in LUSC and PCT. C. miR-493-3p expression in NSCLC and PCT. Overall survival curve of miR-493-3p expression in D.LUAD patients, E. LUSC patients, F. NSCLC patients.

FIG.3

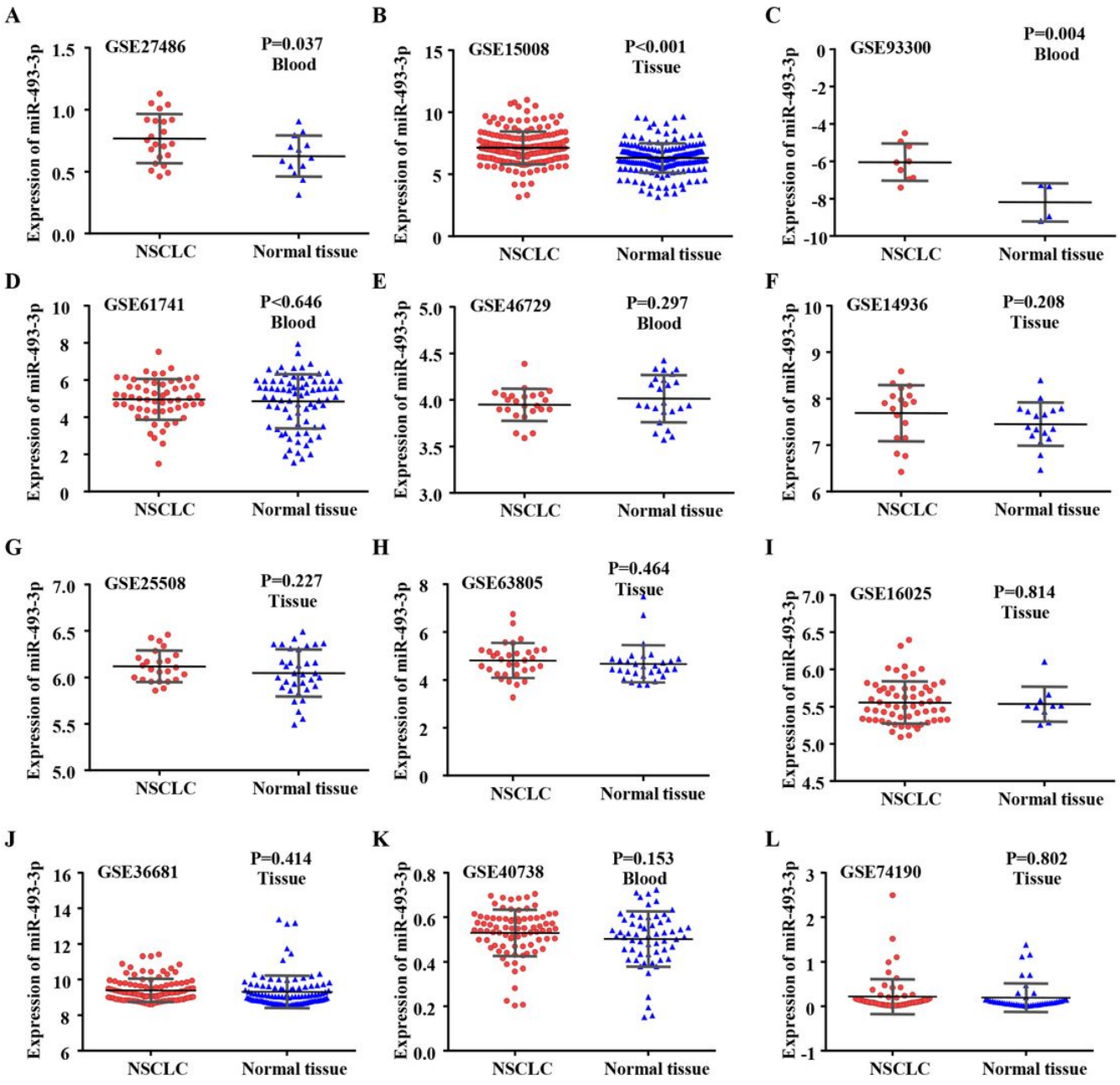

Figure 3

Expression of miR-493-3p in NSCLC patients based on GEO datasets. A. GSE27486, B. GSE15008, C. GSE93300, D. GSE61741, E. GSE46729, F. GSE14936, G.GSE25508, H. GSE63805, I. GSE16025, J. GSE36681, K. GSE40738, L. GSE74190.

FIG.4

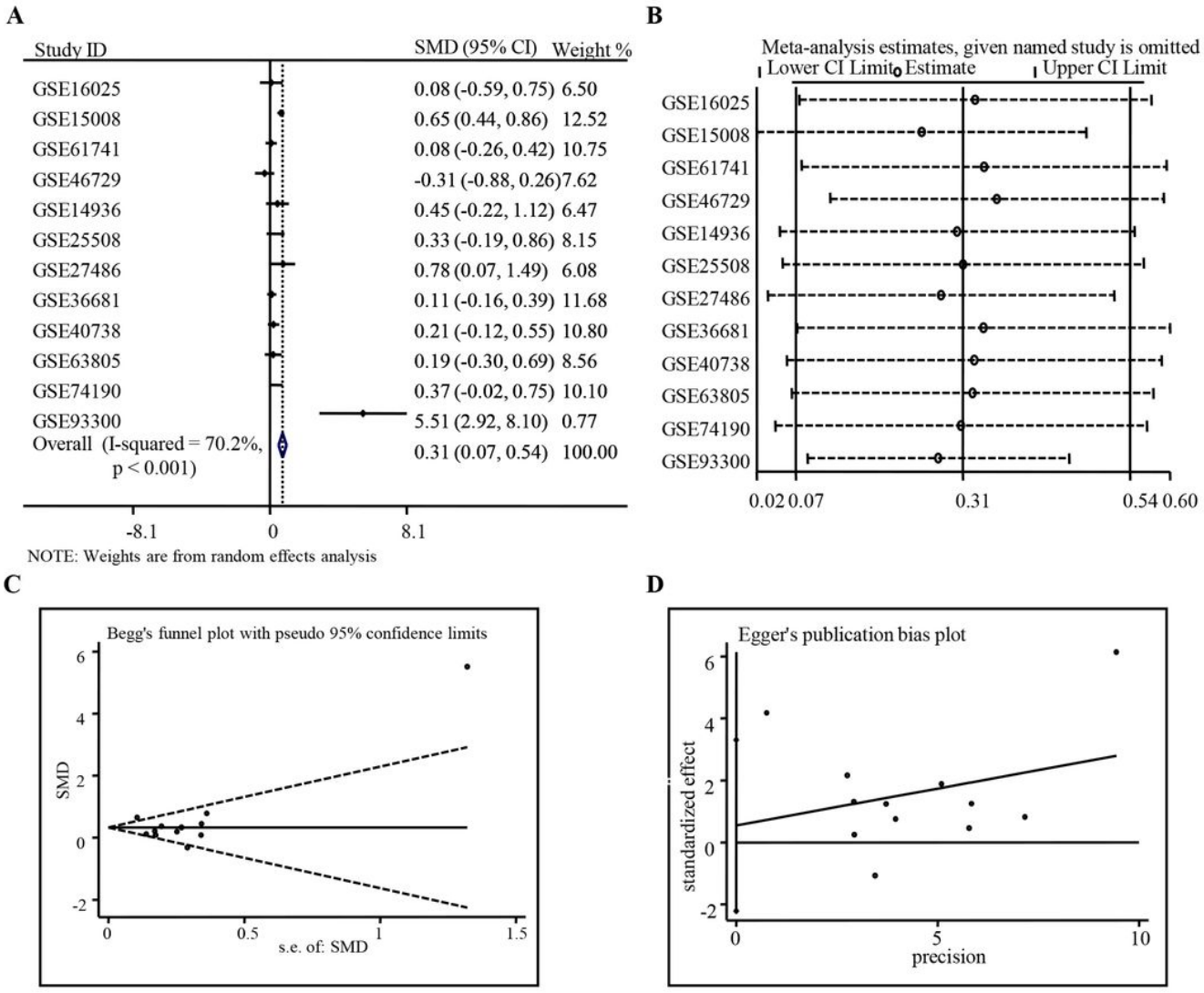

Figure 4

Meta-analysis of miR-493-3p expression in GEO database. A. Forest plot of GEO chips. The pooled standard mean deviation of 0.31 (95%CI: 0.07, 0.54) with great heterogeneity (I2 = 70.2%, p <0.05). B. Sensitivity analysis of GEO chips. C.A funnel plot was applied to evaluate the publication bias of GEO chips (Begg's test, p = 0.15). D.A funnel plot was applied to evaluate the publication bias of GEO chips (Egger's test, p = 0.44).

FIG.5

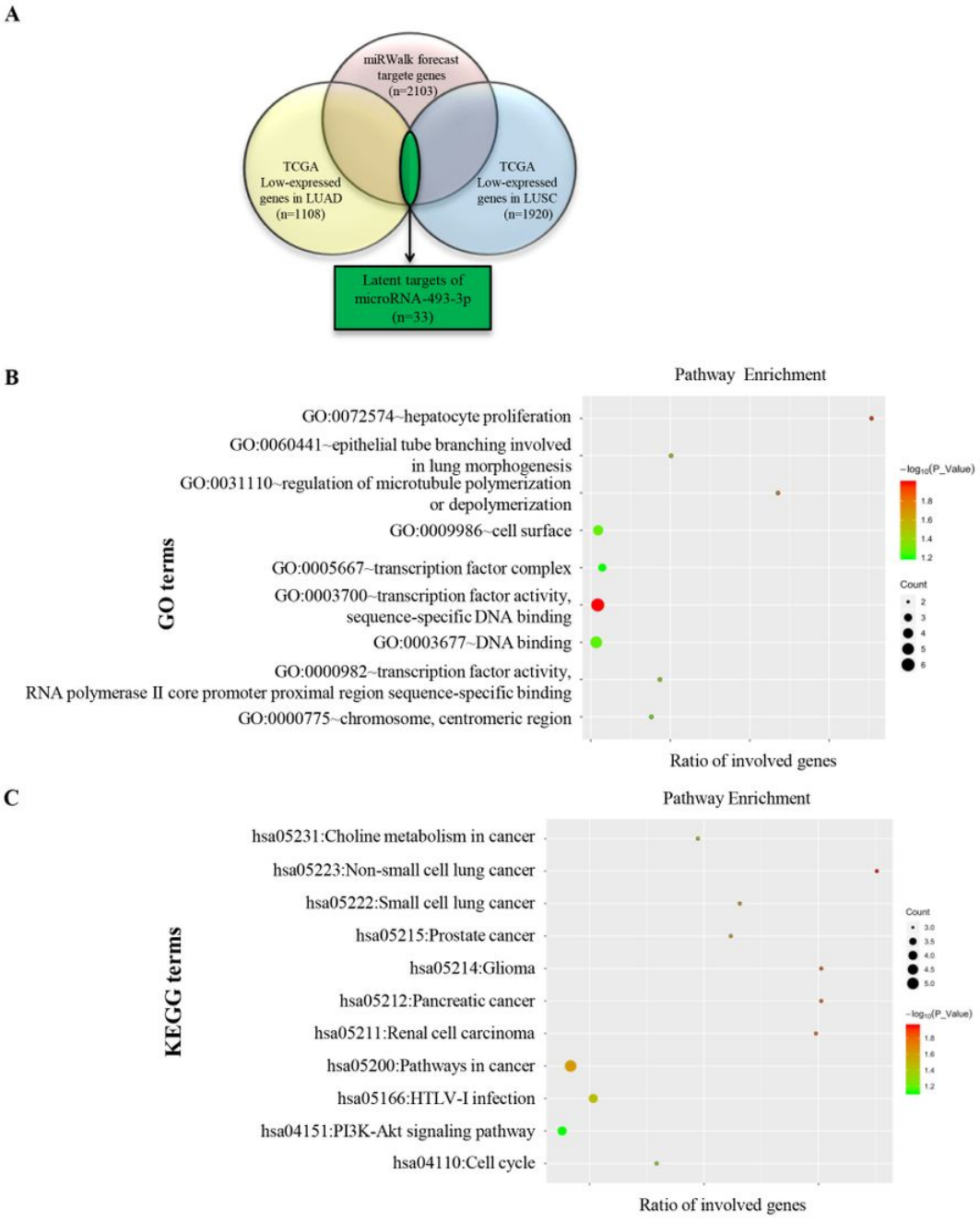

Figure 5

Collection the potential target genes of miR-493-3p, performing GO and KEGG enrichment analysis. A. Venn diagrams of potential target genes of miR-493-3p, showing the intersection (n=33) between different groups, miRWalk2.0 forecast target genes (n=2103) and TCGA low-expressed genes in LUAD (n=1108) and in LUSC (n=1920). B. GO enrichment analysis. C. KEGG enrichment analysis. The x-axis represents the ratio of involved genes, and the y-axis represents the GO and KEGG terms. Each bubble represents a term. The size of the bubble indicates the number of involved genes. Lighter colors indicate smaller P values.

FIG.6

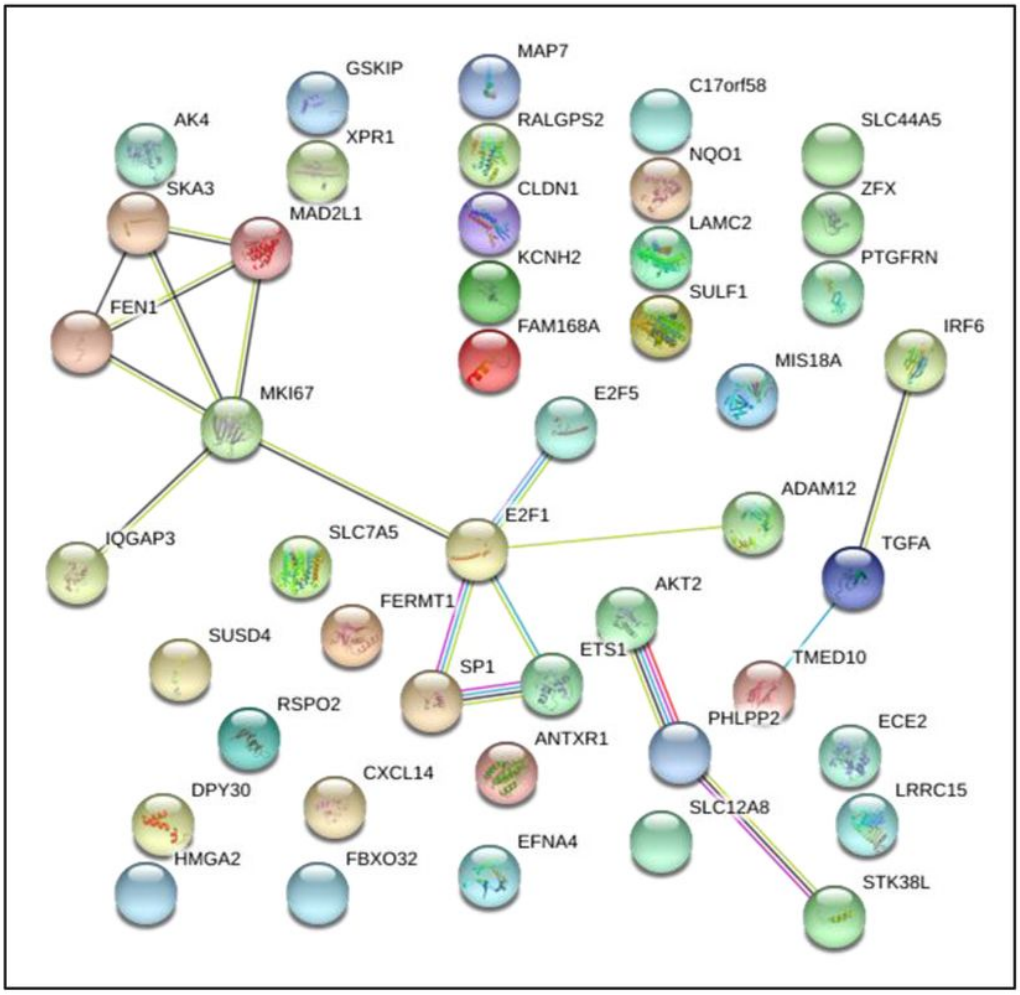

Figure 6

The PPI network of possible miR-493-3p target genes in NSCLC. Each node represents a gene-encoded protein, while lines between the nodes represent protein associations.

FIG.7

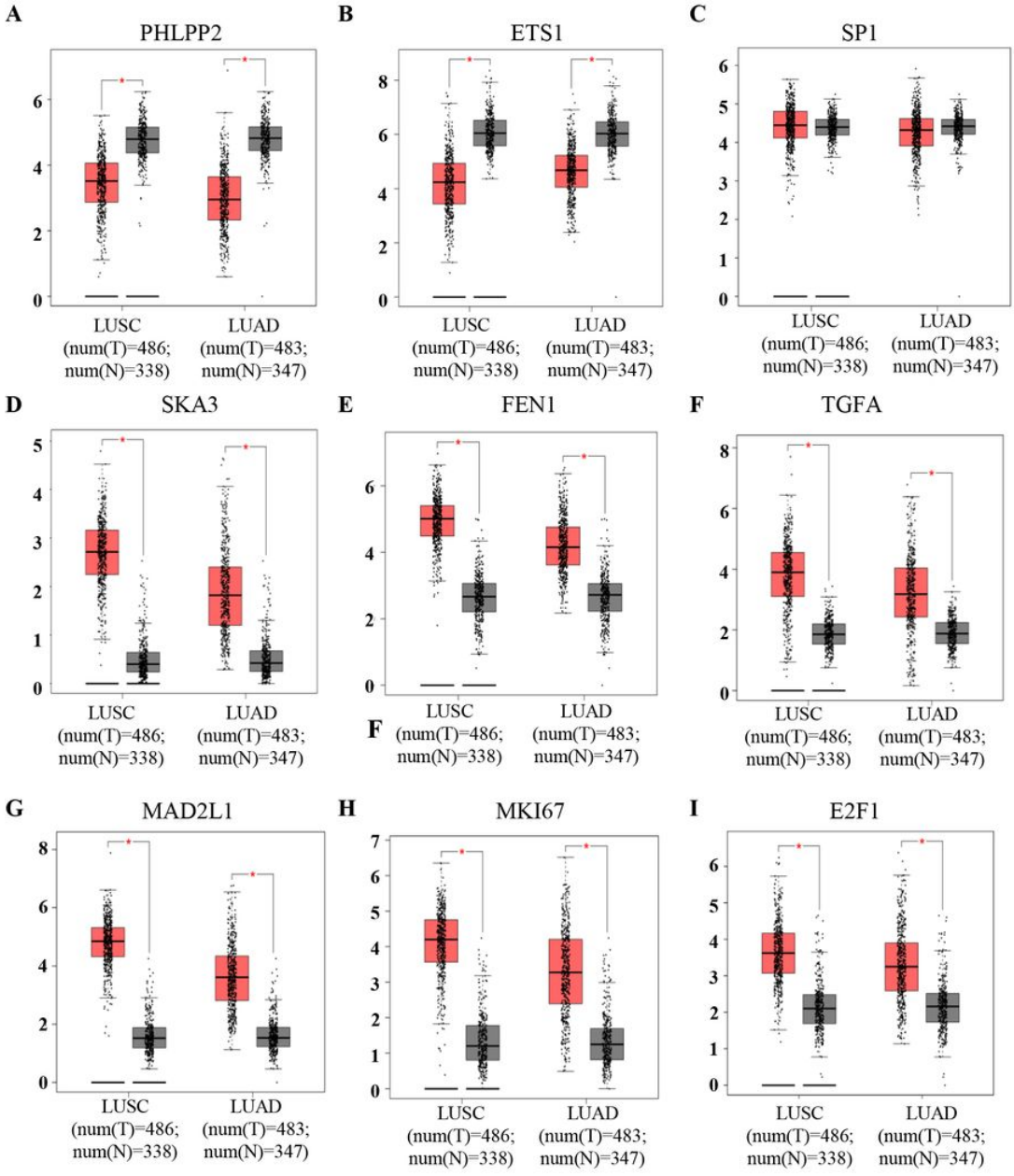

Figure 7

Expression of nine hub genes in LUSC (T, n=486) and normal tissues (N, n=338), LUAD (T, n=483) and normal tissues (N, n=347) based on GEPIA. A. PHLPP2, B. ETS1, C. SP1, D. SKA3, E. FEN1, F. TGFA, G.MAD2L1, H.MKI67, I. E2F1.

FIG.8

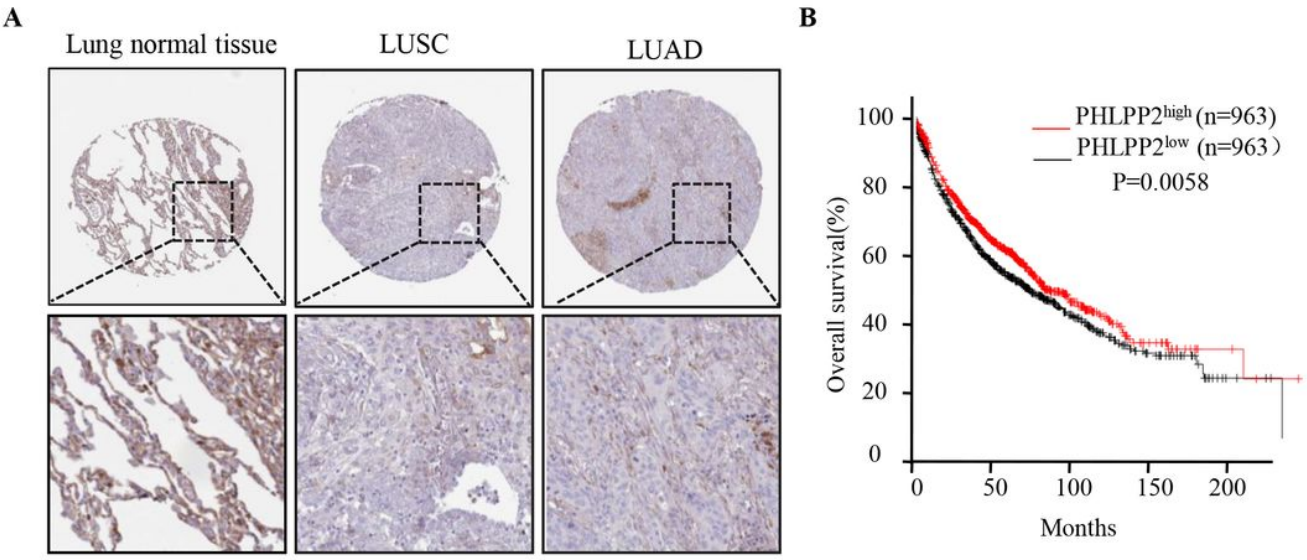

Figure 8

Protein expression and Kaplan-Meier survival curves of PHLPP2. A. Median staining of PHLPP2 in lung normal tissue, LUAD, LUSC (up: magnification of 4×10, down: 4×100). B. OS survival curve of NSCLC patients based on PHLPP2 expression level (P = 0.0058).