Expression and Gene Regulation Network of *NUDT21* in Lung Adenocarcinoma and Prediction of Anticancer Components of Pinellia Ternata Based on Data Mining

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Research

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Abstract

Background

NUDT21 belongs to NUDT families, which is thought to play an essential role in cancer growth and progression in recent years. Abnormal *NUDT21* expression is closely related to lung adenocarcinoma (LUAD). However, the expression level, gene regulation network, and prognostic value of *NUDT21* in LUAD remain unclear. Besides, the active compounds of *Pinellia ternata* against LUAD are still not clear yet. Therefore, an in-depth study of the expression and gene regulation network of *NUDT21* is of great theoretical significance and clinical demand for discovering new targets and strategies for the treatment of LUAD and the further improvement of the therapeutic effect of LUAD. Also, the targeted NUDT21 active ingredients of *Pinellia ternata* were sought to provide a theoretical basis for its clinical application in the treatment of LUAD.

Methods

A variety of online analysis tools were used in this study, including cBioPortal, ONCOMINE, GeneMANIA, GEPIA, Metascape, UALCAN, LinkedOmics, Metascape, TIMER, TRRUST, The Human Protein Atlas, TCMSP, and AutoDock Vina.

Results

The levels of transcription and expression of *NUDT21* were significantly increased in patients with LUAD. Gene altered of *NUDT21* was up to 12% in LUAD patients. However, the promoter methylation level of *NUDT21* in LUAD was lower compared to normal human. LUAD patients' survival with the low expression level of *NUDT21* was better prognostic value than LUAD patients with high expression level. Forty-eight nodes and 572 edges were found in the PPI network constructed with *NUDT21* and its neighboring genes. Regulatory region DNA binding, transcription regulatory region DNA binding, and regulatory region nucleic acid binding were the primary function of *NUDT21* and its neighboring genes. The KEGG pathway of *NUDT21* and its neighboring genes were mainly involved in the apelin signaling pathway, PI3K-Akt signaling pathway, and axon guidance. Our results showed that DNMT1, HDAC1, and MYC were the critical transcription factor targets involved in the network of *NUDT21* and its neighboring genes. We also found that CDK1, ATM and PLK1 were main kinase targets in the *NUDT21* kinase-target network. The *NUDT21* miRNA-target network was associated with MIR-302C, MIR-9, and MIR-330. Moreover, the expression of *NUDT21* was positively related to the infiltration of CD8 + T cells, macrophages, neutrophils and dendritic cell. 13 active compounds of *Pinellia ternata* were retrieved from the TCMSP. Among them, baicalein was the best combination with NUDT21.

Conclusions

Our results revealed the expression and potential regulatory network of *NUDT21* in LUAD, laying a foundation for further research on the role of *NUDT21* in cancer. Furthermore, we offer new therapeutic targets and prognostic biomarkers for the reference. Finally, we provide potential therapeutic drugs from traditional Chinese medicine in the treatment of LUAD.

1. Introduction

Lung cancer is one of the deadliest malignancies in the world [1]. According to statistics, the number of lung cancer deaths was as high as 1.8 million, accounting for 18.4% [2]. Moreover, accounts for about 85% of lung cancer are non-small cell lung cancer, three histological subtypes that include squamous carcinoma, large cell undifferentiated carcinoma, and adenocarcinoma [3]. Among them, lung adenocarcinoma (LUAD) is the most common form of lung cancer. The pathogenesis of LUAD is extraordinarily complex and has not been fully elucidated. Studies have shown that many factors were involved in the development of LUAD, including diet, smoking,and genetic susceptibility[4–6]. Treatment for LUAD has progressed from the initial surgery, radiotherapy and chemotherapy to molecular-targeted therapy and immune-targeted therapy [7, 8]. The innovations in the treatment of LUAD might significantly prolong the overall survival benefit of patients, but the survival prognosis for LUAD is still low. Part of the reasons for this mainly was associated with unknown target localization, difficulty in early diagnosis, high risk of cancer recurrence and high rate of early metastasis [9]. However, the discovery of various subtypes of lung cancer targets and the introduction of targeted therapy has changed the prognosis of patients with lung cancer by incorporating tumor genotyping into treatment decisions [10]. The results from the European EUHER2 showed that non-small-cell lung cancer patients, a known HER2 exon-20 insertion, treated with HER2-targeted drugs had established an excellent therapeutic effect on lung cancer [11]. Therefore, targeted therapy is expected to become the crucial methods of treating LUAD in the future, and bring good news for patients with LUAD.

NUDT21, as a member of NUDT families, that existed in nearly all organisms. And also is a tumor suppressor gene in the progression of cancer [12]. To date, its biological function remains unclear. *NUDT21* as mRNA precursor 30-end modification factor mainly regulated 30UTR shortening. It participated in the normal physiological process of proliferation, differentiation and apoptosis in cells [13]. Previous studies had found that *NUDT21* played an essential role in cancer growth and progression [14, 15]. *NUDT21* could inhibit various tumor growths, including glioblastoma, breast cancer, and cervical cancer [16–18]. However, the overexpression of *NUDT21* was found in multiple cancers, including hepatocellular carcinoma, and leukemia [19, 20]. However, we found that the overexpression and gene regulation network of *NUDT21* in patients with LUAD by clinical trial data integration. Therefore, *NUDT21* may be potential therapeutic targets and prognostic biomarkers for patients with LUAD.

Pinellia ternata, a plant of the Araceae has been used with its dried tuber as a drug in China for thousands of years. *Pinellia ternata* properties were spicy, warm, toxic, return to the spleen, stomach, and lung meridian. It had been documented by the Chinese Pharmacopoeia (2015 edition) as a commonly used Chinese medicine for the treatment of infection, inflammation, cough, and vomiting [21, 22].

Excepted for the pharmacological effects of anti-asthmatic, anti-tussive, anti-inflammatory anti-emetic, and sedative-hypnotic, in recent years, more and more studies have found that *pinellia ternata* has a therapeutic effect on various cancer [23, 24]. To date, few studies have reported whether *Pinellia ternata* have potential therapeutic effects on LUAD.

In this study, to identify the overexpression and gene regulation network of *NUDT21* in LUAD, we used the Cancer Genome Atlas (TCGA) and various public databases to explore the expression and differences of *NUDT21* in LUAD patient. Moreover, we also explore the potential active components of *Pinellia ternata* in the treatment of LUAD by using network pharmacology and molecular docking methods. Finally, we hope that this approach will provide a new idea for the target treatment of other disease and screen the potential active ingredients of *Pinellia ternata* as a potential new drug against LUAD.

1. Materials And Methods

1.1 Oncomine analysis

Oncomine (www.oncomine.org) is a large tumor gene chip database that provides translational bioinformatics services for scientific researcher [25]. In our study, we registered platform of Oncomine database, and set the screening criteria as follow: (1) Gene: *NUDT21*; (2) Cancer type: lung adenocarcinoma; (3) Analysis type: cancer vs normal analysis; (4) Data type: mRNA; (5) Threshold setting conditions: $P = 0.05$, fold change = 2, and gene rank = top 10%. Student's *t*-test was used to analyze the difference of *NUDT21* expression in LUAD.

1.2 UALCAN analysis

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a portal for tumor subgroup gene expression and survival analyses [26]. We choose “Expression Analysis” module of UALCAN to analyst TCGA gene expression in this study, and the screening criteria were set as follow: (1) Gene: *NUDT21*; (2) Dataset: lung adenocarcinoma. (3) Threshold setting conditions: *P*-value cutoff = 0.05. Student’s *t*-test was used for comparative analysis.

1.3 The Human Protein Atlas analyses

The Human Protein Atlas (https://www.proteinatlas.org/) is a platform that provides cells, tissues and organs distribution information on all 24,000 human proteins and offers free public access. In this study, we analyzed the protein expression levels of *NUDT21* in the lung tissue of patients with AULD.

1.4 GEPIA analysis

GEPIA (http://gepia.cancer-pku.cn/index.html) is a platform that is analyzing the RNA sequencing expression data of 9,736 tumours and 8,587 normal samples from the TCGA and the GTEx projects [27]. A variety of analytical methods were conducted in this study, including differential mRNA expression analysis, pathological stage analysis, and correlative prognostic analysis. We set the screening criteria as follow: (1) Gene: *NUDT21*; (2) Dataset: LUAD; (3) Threshold setting conditions: *P*-value cutoff = 0.05.

Student's *t*-test was used to analyze the expression of *NUDT21* or pathological stage of LUAD. The Kaplan-Meier curve was used to analyze the prognosis of LUAD.

1.5 cBioPortal analysis

The cBioPortal (http://cbioportal.org) is an open-source for interactive exploration of multidimensional cancer genomics datasets that provide a visualization tool for studying and analyzing cancer genetic data [28]. In our study, genetic alterations and coexpression of *NUDT21* were conducted from cBioPortal. The screening criteria were set as follow: (1) 230 samples of lung adenocarcinoma were analyzed; (2) mRNA expression z scores relative to all samples (log RNA Seq V2 RSEM) were obtained using a z score threshold of ± 2.0. (3) Gene: *NUDT21*.

1.6 STRING analysis

STRING (https://string-db.org/cgi/input.pl) is a platform used to construct protein-protein interaction networks between target proteins [29]. This study builds the PPI network interaction by screening condition with medium confidence (0.4) and defined species as Homo sapiens.

1.7 GeneMANIA analysis

GeneMANIA (http://www.genemania.org) is a network for building protein-protein interactions (PPI), generating hypotheses about gene function, analyzing gene lists, and sequencing genes for function determination [30]. In this study, we constructed interaction networks for analyses the role of *NUDT21* and the top 50 neighbor altered gene.

1.8 Metascape analysis

Metascape (https://metascape.org) is a simple and powerful gene function annotation and analysis tool that can help users apply the currently popular bioinformatics analysis methods to analyze batch genes and proteins to realize the knowledge of gene or protein function [31]. In our study, GO function and KEGG pathways enrichment analysis of *NUDT21* and the top 50 neighbors' altered gene in LUAD were analyzed using Metascape.

1.9 TRRUST analysis

TRRUST is a manually curated database of human and mouse transcriptional regulatory networks that contain 8,444 and 6,552 TF-target regulatory relationships of 800 human TFs and 828 mouse TFs, respectively [32]. In this study, we tried to determine the key regulated factor of *NUDT21* and the top 50 neighbors altered gene in LUAD by using TRRUST.

1.10 LinkedOmics analysis

LinkedOmics (http://www.linkedomics.org/) is a publicly available platform that includes multi-omics data from all 32 TCGA Cancer types [33]. It provides methods for analyzing and comparing cancer multi-omics data within and across tumour types. In our study, kinase target enrichment, miRNA target enrichment, and genes differentially expressed in correlation with *NUDT21* were conducted by using the

“LinkInterpreter” module. The screening criteria set as follow: (1) a minimum number of genes (size) of 3; (2) cancer type: LUAD; (3) a simulation of 500; (4) search attribution: *NUDT21* and the top 50 neighbors altered gene. (5) Target dataset: RNAseq (data type).

1.11 Timer analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for systematical tumor immune analysis, including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells [34]. In our study, the correlation among clinical outcome and *NUDT21* expression and the infiltration of immune cells was evaluated by “Survival module”. The correlation between *NUDT21* expression level and the infiltration of immune cells was assessed by “Gene module”.

1.12 TCMSP analysis

TCMSP (http://lsp.nwu.edu.cn/tcmsp.php) is a pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets and diseases [35]. In this study, the active ingredient of *Pinellia ternata* with OB≥30% and DL≥0.18 were obtained.

1.13 Molecular docking analysis

AutoDock Vina (http://vina.scripps.edu/) is an open-source program for doing molecular docking, which significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4 [36]. Protein Database Bank (PDB, https://www.rcsb.org/) was used to acquire the protein crystal structure of target genes (*NUDT21*). 3D structures of active compounds were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/). Vina software 1.1.2 was used for molecular docking. Finally, the conformation with the lowest score was selected and plotted with PyMOL 2.4 for analysis.

2. Results

2.1 *NUDT21* expression in LUAD

Compared the transcription levels of *NUDT21* in lung tissue of patients with LUAD and normal human with the ONCOMINE, we found that the transcriptional levels of *NUDT21* were significantly increased in patients with LUAD (Fig. 1). Moreover, these results were similar to Table 1. It showed that the mRNA level of *NUDT21* was increased in LUAD than normal human. Yamagata N’s dataset found that the transcriptional level of *NUDT21* (fold change = 2.417 and $p = 0.008$) in LUAD was significantly increased [37]. Analogously, the transcriptional levels of *NUDT21* in LUAD were significantly up-regulated in datasets of Okayama H (fold change = 1.841 and $p = 7.96E-17$) [38]. The fold change of *NUDT21* expression in LUAD was 1.852 (p = 2.49E-4) in Landi MT datasets [39].

Table 1
The mRNA levels of *NUDT21* in different types of LUAD tissues and normal lung tissues at transcriptome level (ONCOMINE).

TLR	Type	Fold change	*P*value	*t*test	Reference
NUDT21	LUAD	2.417	0.008	2.966	(1)
	LUAD	1.841	7.96E-17	11.637	(2)
	LUAD	1.852	1.72E-14	8.884	(3)

Furthermore, we also compared the expression levels of *NUDT21* in LUAD tissue and normal tissues with the UALCAN. Our results showed that the transcriptional levels of *NUDT21* stratified based on gender and stages ($P = 0.05$) in LUAD tissues were significantly up-regulation (Fig. 2). The relative expression level of *NUDT21* in LUAD tissues was evaluated with the GEPIA. The results showed that the relative expression level of *NUDT21* was increased (Fig. 3). Besides, we compared the protein expression levels of *NUDT21* in lung tissue of PAAD patients and normal people with the Human Protein Atlas. We found that the protein expression levels of *NUDT21* were up-regulated in LUAD (Fig. 4). Then, the correlation between differential expression of *NUDT21* and pathological stage in patients with LUAD was assessed. Our results showed a significant correlation between the expression of *NUDT21* and pathological stage ($P = 0.00106$) (Fig. 5). Moreover, we evaluated the prognostic value of *NUDT21* in LUAD patients with GEPIA. The results showed that LUAD patients' survival with the low expression level of *NUDT21* was better prognostic value than LUAD patients with a high expression level of *NUDT21* ($P = 0.0029$)(Fig. 6).

2.2 Interaction network of *NUDT21* alterations in LUAD

The interaction network of *NUDT21* on the molecular characteristics in LUAD was analysis. First, the genetic alteration of *NUDT21* was evaluated with the TCGA. We found that *NUDT21* were altered by 12% (Fig. 7A). However, the promoter methylation level of *NUDT21* in LUAD was lower compared to normal human (Fig. 7B). Moreover, we found of NUDT21-neighboring genes (the 50 most frequently altered neighbor genes) that were altered at frequencies > 17% in LUAD (Table 2). The most frequent alterations among the NUDT21-neighboring genes were *TTN2* (64.29%), *TP53* (60.71%), and *MUC16* (57.14%). We then explore the potential interactions of *NUDT21*, and its neighboring genes, the PPI network analysis of them was established with STRING. We found that 48 nodes and 572 edges were obtained in the PPI network (Fig. 7C). We also found that regulatory region DNA binding, transcription regulatory region DNA binding, regulatory region nucleic acid binding, and regulation of homeostatic process were the primary function of *NUDT21* and its neighboring genes with the results of GeneMANIA (Fig. 7D).

Table 2
The top 50 of *NUDT21* neighbor gene alterations in LUAD (cBioPortal).

Gene	Altered group	Unaltered group	*P*-value
TTN2	18 (64.29%)	89 (44.06%)	0.0351
TP53	17 (60.71%)	90 (44.55%)	0.0802
MUC16	16 (57.14%)	75 (37.13%)	0.0353
FLG	12 (42.86%)	50 (24.75%)	0.0398
RYR2	12 (42.86%)	70 (34.65%)	0.259
CSMD3	11 (39.29%)	70 (34.65%)	0.388
ZFHX4	10 (35.71%)	53 (26.24%)	0.202
LRP1B	10 (35.71%)	58 (28.71%)	0.289
NELL1	8 (28.57%)	13 (6.44%)	1.220e-3
ZNF804A	8 (28.57%)	30 (14.85%)	0.0651
ZNF536	8 (28.57%)	34 (16.83%)	0.109
KMT2C	8 (28.57%)	35 (17.33%)	0.123
KRAS	8 (28.57%)	67 (33.17%)	0.400
USH2A	8 (28.57%)	60 (29.70%)	0.548
FBN2	7 (25.00%)	25 (12.38%)	0.0709
DMD	7 (25.00%)	26 (12.87%)	0.0822
ADAMTS12	7 (25.00%)	31 (15.35%)	0.154
COL11A1	7 (25.00%)	33 (16.34%)	0.190
SGIP1	6 (21.43%)	8 (3.96%)	2.894e-3
KMT2D	6 (21.43%)	13 (6.44%)	0.0167
ZNF521	6 (21.43%)	17 (8.42%)	0.0434
TRPS1	6 (21.43%)	19 (9.41%)	0.0635
LRP2	6 (21.43%)	20 (9.90%)	0.0754
CSMD2	6 (21.43%)	22 (10.89%)	0.103
TAF1L	6 (21.43%)	24 (11.88%)	0.135
TNR	6 (21.43%)	25 (12.38%)	0.153

RELN	6 (21.43%)	29 (14.36%)	0.235
RYR1	6 (21.43%)	29 (14.36%)	0.235
ANK2	6 (21.43%)	30 (14.85%)	0.258
HMCN1	6 (21.43%)	30 (14.85%)	0.258
FLG2	6 (21.43%)	31 (15.35%)	0.281
PAPPA2	6 (21.43%)	33 (16.34%)	0.330
CSMD1	6 (21.43%)	38 (18.81%)	0.455
FAT3	6 (21.43%)	38 (18.81%)	0.455
PCLO	6 (21.43%)	40 (19.80%)	0.504
MUC17	6 (21.43%)	42 (20.79%)	0.553
CAMTA1	5 (17.86%)	2 (0.99%)	3.364e-4
CHD9	5 (17.86%)	5 (2.48%)	3.088e-3
OR2L2	5 (17.86%)	6 (2.97%)	5.177e-3
KPRP	5 (17.86%)	8 (3.96%)	0.0121
FRMPD1	5 (17.86%)	9 (4.46%)	0.0171
USP29	5 (17.86%)	9 (4.46%)	0.0171
HERC2	5 (17.86%)	12 (5.94%)	0.0405
BCORL1	5 (17.86%)	13 (6.44%)	0.0513
CDH7	5 (17.86%)	13 (6.44%)	0.0513
TRPM6	5 (17.86%)	13 (6.44%)	0.0513
CCDC178	5 (17.86%)	14 (6.93%)	0.0636
CTNND2	5 (17.86%)	14 (6.93%)	0.0636
EPHA3	5 (17.86%)	14 (6.93%)	0.0636
HECW1	5 (17.86%)	15 (7.43%)	0.0775

2.3 Gene ontology (GO) and KEGG pathway enrichment analysis

The GO function and KEGG pathways enrichment analysis of *NUDT21* and the top 50 neighbors altered gene in LUAD was explored with the Metascape. Our results showed that the cellular components related to *NUDT21* and its neighboring genes mainly involved in the extracellular matrix, apical plasma

membrane, z disc, cell cortex, and postsynapse (Fig. 8A). Moreover, the release of sequestered calcium ion into cytosol by sarcoplasmic reticulum, cardiac chamber development, and protein tetramerization were the main biological processes (Fig. 8B). The molecular functions of *NUDT21* and the top 50 neighbors altered gene were mainly included calcium ion binding, structure molecule activity, and protein domain specific binding (Fig. 8C). The KEGG pathway of *NUDT21* and its neighboring genes were mainly involved in the apelin signaling pathway, PI3K-Akt signaling pathway, and axon guidance (Fig. 8D). We also found that non-small cell lung cancer recurrent, mixed tumor and sarcomatoid renal cell carcinoma was the manly disease related to *NUDT21* and its neighboring genes (Fig. 8E).

2.4 Transcription factor targets, kinase targets, and miRNA targets of *NUDT21* in LUAD

The potential transcription factor targets, kinase targets and miRNA targets of *NUDT21* in LUAD were obtained in the database of TRRUST and LinkedOmics. The results showed that DNA (cytosine-5-)-methyltransferase 1 (DNMT1), histone deacetylase 1(HDAC1), and v-myc myelocytomatosis viral oncogene homolog (MYC) were the key transcription factor targets involved in the network of *NUDT21* and its neighboring genes (P 0.05) (Table 3). We also found that the top three kinase targets and miRNA targets of the NUDT21 network with the LinkedOmics (Table 4). Kinase CDK1, Kinase ATM and Kinase PLK1 were the top three targets in the *NUDT21* kinase-target network (P 0.00). The *NUDT21* miRNA-target network was associated with (ATGTTAA) MIR-302C, (TAGCTTT) MIR-9, and (TGCTTTG) MIR-330 (P 0.00).

Table 3
Key regulated factor of *NUDT21* and the top 50 neighbor altered gene in LUAD (TRRUST).

Key TF	Description	Regulated gene	*P*-value	FDR
DNMT1	DNA (cytosine-5-)-methyltransferase 1	RELN, TP53	0.00293	0.0146
HDAC1	histone deacetylase 1	RELN, TP53	0.0146	0.0366
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	KRAS, TP53	0.0278	0.0464

Table 4
The Kinase target and miRNA target of NUDT21 in LUAD (LinkedOmics).

Cancer type	Enriched Category	Gene set	Leading Edge Number	*P*-value	FDR
LUAD	Kinase Target	Kinase CDK1	104	0	0
		Kinase ATM	55	0	0
		Kinase PLK1	38	0	0
	miRNA Target	ATGTTAA,MIR-302C	77	0	0
		TAGCTTT,MIR-9	82	0	0
		TGCTTTG,MIR-330	114	0	0

2.5 The correlation of genes differentially expressed and *NUDT21* in LUAD

The mRNA sequencing data from 515 patients with LUAD in the TCGA were obtained from the LinkedOmics. As shown in Fig. 9A, the results showed that 19988 genes were closely related to *NUDT21*. Among them, 11442 genes showed remarkable positive correlations with *NUDT21* ($P < 0.05$), while 4387 genes showed significant negative correlations ($P < 0.05$). We also showed the 50 significant genes positively and negatively correlated with *NUDT21* in LUAD (Fig. 9B, 9C). Moreover, the expression of *NUDT21* had an intense positive association with OGFOD1 (Pearson correlation = 0.5997, $P = 1.301e{-}51$) (Fig. 10A), RSPRY1 (Pearson correlation = 0.5632, $P = 8.081e{-}48$) (Fig. 10B), CNOT1 (Pearson correlation = 0.5632, $P = 1.944e{-}44$) (Fig. 10C).

2.6 Immune cell infiltration of *NUDT21* in LUAD

To further reveal the relationship between the immune cell infiltration and *NUDT21* in LUAD, we conducted the correlation of them with the TIMER. The expression of *NUDT21* was positively associated with the infiltration of CD8 + T cells (Cor = 0.309, $P = 3.06e\text{-}12$), macrophages (Cor = 0.173, $P = 1.23e-4$), neutrophils (Cor = 0.267, $P = 2.47e-9$) and dendritic cell (Cor = 0.233, $P = 25e-7$) (Fig. 11).

2.7 Active compounds of *Pinellia ternata* and molecular docking

A total of 116 compounds were found in TCMSP database. Among them, 13 active compounds were retrieved base on OB and DL in homeostatic process (Table 5). Besides, to verify the binding ability of active compounds of *Pinellia ternata* to NUDT21, we conducted molecular docking with the AutoDock Vina. In this analysis, the vina score's value (binding energy) indicates the binding activity between a protein and a compound. The binding energy ≥ -5.0 kcal/mol was considered the compound and protein with a suitable binding property. Our results showed that baicalein was the best combination with

NUDT21 (Score =-8.8 kcal/mol) (Fig. 12A). Van der Waals, Pi- cation, conventional-hydrogen bond, Pi-Pi stacked, carbon hydrogen bond, unfavorable donor-donor, and Pi- alkyl were the mainly interacted mode between them (Fig. 12B).

Table 5
The results of screening active components of *Pinellia ternata* by TCMSP database.

MOL ID	MOL Name	OB(%)	DL
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76
MOL002670	Cavidine	35.64	0.81
MOL002714	baicalein	33.52	0.21
MOL002776	Baicalin	40.12	0.75
MOL000358	beta-sitosterol	36.91	0.75
MOL000449	Stigmasterol	43.83	0.76
MOL005030	gondoic acid	30.7	0.2
MOL000519	coniferin	31.11	0.32
MOL006936	10,13-eicosadienoic	39.99	0.2
MOL006937	12,13-epoxy-9-hydroxynonadeca-7,10-dienoic acid	42.15	0.24
MOL006957	(3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl)piperazine-2,5-quinone	46.89	0.27
MOL003578	Cycloartenol	38.69	0.78
MOL006967	xanthine-9	44.72	0.21

3. Discussion

NUDT21, as a tumor suppressor gene,which could inhibit the occurrence and development of various cancers, including small cell lung cancer, bladder cancer, and breast cancer. *NUDT21* is a 3'-terminal processing factor of mRNA precursor (targeted mRNA precursors 3'contain multiple poly-A splicing sites), regulating mRNA expression through selective alternative polyadenylation [40]. Ultimately, the normal physiological process of *NULDT2*1 was involved in regulating proliferation, differentiation and apoptosis. However, the overexpression of tumor suppressor genes in cancer patients has attracted more and more attention. But the mechanism of them has not yet been elucidated. Studies had found that it might be closely related to genetic alters and DNA methylation [41, 42]. However, the expression and gene regulation network of *NUDT21* in patients with LUAD was rarely reported.

In our study, the expression level of *NUDT21* and the correlation between differential expression of *NUDT21* and pathological stage were first explored in LUAD patients. We found that *NUDT21* was up-

regulated expression in patients with LUAD compared with normal human. In addition, our results showed a significant correlation between the expression of *NUDT21* and pathological stage. Furthermore, the survival of LUAD patients with the low expression level of *NUDT21* was better prognostic value than LUAD patients with a high expression level of *NUDT21*. In brief, overexpression of *NUDT21* may play an essential role in survival with LUAD patients. Contrary to other studies, *NUDT21* was low expressed in varies cancer [43]. To further explore the mechanism of *NUDT21* overexpression in LUAD patients, we found that genetic alteration of *NUDT21* was high (12%). Moreover, the promoter methylation level of *NUDT21* in LUAD was lower compared to normal human. Therefore, genetic alteration and methylation of *NUDT21* may be the leading cause of *NUDT21* overexpression. Moreover, NUDT21-neighboring genes (the 50 most frequently altered neighbor genes) were altered at frequencies > 17% in LUAD. We then explore the potential interactions and function of *NUDT21* and its neighboring genes. We found that *NUDT21* and its neighboring genes have complex and tight networks of connections. Their primary functions mainly include the regulatory region DNA binding, transcription regulatory region DNA binding, and regulatory region nucleic acid binding. The above evidence reveals that they might affect the processes of gene binding, transcription, and regulation involved in the occurrence and development of cancer.

Moreover, GO enrichment analysis revealed that these genes' functions are mainly connected with inflammatory response caused by the activation of inflammatory factors. As expected, the expression of *NUDT21* was positively associated with the immune cell infiltration, including CD8 + T cells, macrophages, neutrophils and dendritic cell. Immunotherapy has been recognized as the fourth pillar of cancer therapy, and a large number of preclinical and clinical studies have been revealed the efficacy of immunotherapy for lung cancer [44]. Targeting and regulating the tumor immune microenvironment can divert the immune system to anticancer and increase the sensitivity to established chemotherapy. Chemokines and their receptors are crucial for inflammation and anti-tumor immunity, thus influencing angiogenesis, tumor occurrence, progression, metastasis, therapeutic efficacy, and patient outcomes [45–47]. Studies have shown that the CXC family of chemokines and their receptors are associated with tumour metastasis and therapy resistance [48, 49]. Besides, our results showed that The KEGG pathway of *NUDT21* and its neighboring genes involved in the apelin signaling pathway, PI3K-Akt signaling pathway, and axon guidance. Apelin is an endogenous ligand of G protein coupled receptor APJ. It is widely expressed in many tissues, especially the lungs. More and more evidence revealed that the apelin signaling pathway is closely associated with the occurrence of respiratory diseases [50]. Studies have found that inhibiting apelin effectively remodeled the tumor microenvironment, reduced angiogenesis, and effectively inhibited tumor growth [51]. Importantly, apelin prevents resistance to antiangiogenic receptor tyrosine kinase (RTK) inhibitor therapy in lung cancer [51]. PI3K-Akt signaling pathways play a key role in the regulation of cell proliferation, differentiation and apoptosis. AKT is a crucial regulator of cell survival and apoptosis [52, 53]. AKT is activated by PDK1 through phospholipid binding and activation loop phosphorylation at Thr308 [52, 53]. AKT inhibits apoptosis and promotes survival of malignant cells by phosphorylating and inactivating multiple targets, including Bad, C-Raf and Caspase-9 [54]. In recent years, research has shown that the axon guidance cue molecule Slit2 could suppress lung cancer cell invasion and growth [55].

We also focus on their targets and regulators. We then explored the transcription factor targets, kinase targets and miRNA targets of *NUDT21* and its neighboring genes, and found that DNMT1, HDAC1, and MYC were their key regulated factor in LUAD. DNMT1 is a major epigenetic enzyme, maintains the genomic stability and the epigenetic state of DNA by copying CpG methylation markers and generating heritable methylation patterns through cell metabolism [56, 57]. Approaches for inhibition of DNMT1 may become novel strategies for treating cancers [58]. In recent years, abnormal expression of HDAC1 has been found to have potential clinical prognostic value in various cancers. Studies have shown that down-regulation of HDAC1 can inhibit cell proliferation, migration, invasion, angiogenesis and induce cell apoptosis [59]. MYC is a transcription factor that is overexpressed in tumors and participates in preventing immune cells from attacking tumor cells by inducing the expression of PD-L1. MYC expression's role is an alternative marker for evaluation of treatment with non-small cell lung cancer [60]. Therefore, Targeting DNMT1, HDAC1, and MYC may be a promising approach to the treatment of LUAD. We also found that Kinase CDK1, Kinase ATM and Kinase PLK1 were the top three targets in the *NUDT21* kinase-target network. Kinase CDK1, Kinase ATM and Kinase PLK1 are involved in DNA repair and progression through the cell cycle. Kinase ATM and RNF8/RNF168 ubiquitin-ligase modify chromatin extensively on the DNA damaged side. In mitosis, phosphorylation of 53BP1 by Kinase PLK1 and Kinase CDK1 impairs the ability of 53BP1 to bind to ubiquitination H2A and locate DNA damage sites properly [61]. The *NUDT21* miRNA-target network was associated with MIR-302C, MIR-9, and MIR-330. They are involved in tumor proliferation, differentiation, and invasion. It had been reported that Mir-302C-5P act as a cancer suppressor in a variety of cancers [62]. Moreover, Mir-302c-3p is a tumor suppressor gene of malignant glioma and can be used as a new biomarker to predict glioma patients' clinical prognosis [63]. miR-9 includes three family members, including miR-9-1, miR-9-2 and miR-9-3. Researchers recently found that the promoter region of Mir-9-3 was hypermethylated in lung cancer, leading to the down-regulation of Mir-9-3, and poor prognosis of patients and miR-9 may be a potential lung cancer biomarker [64, 65]. MiR-330 acts as a tumor-suppressor microRNA (miRNA) in various cancers and is a promising candidate for miRNA replacement therapy in lung cancer patients [66]. In conclusion, the transcription factor targets, kinase targets and miRNA targets of *NUDT21* and its neighboring genes may be potential therapeutic targets and biomarker for LUAD.

Pinellia ternata is a commonly used Chinese medicine for treating infection, inflammation, cough, and vomiting,which had been documented by the Chinese Pharmacopoeia (2015 edition) [21, 22]. However, more and more studies have found that *Pinellia ternata* has a therapeutic effect on various cancers [23, 24]. Our results found that 13 active compounds of *Pinellia ternata* were retrieved base on OB and DL from the TCMSP. Among them, baicalein was the best combination with NUDT21 (Score =-8.8 kcal/mol). Hydrogen bonding was their main interaction force (Six hydrogen bonds were found in our study). Baicalein was a bioactive component extracted from various traditional Chinese medicines, including *Pinellia ternata* and Scutellaria baicalensis Georgi [67, 68]. In recent years, anti-tumor, anti-inflammatory, and antimicrobial activities of baicalein were found [69]. It had been reported that baicalein could reverse cisplatin resistance of human lung adenocarcinoma A549 cells via PI3K/Akt/NF-κB pathway[70].

Although our study has not been further verified the anti-LUAD of baicalein by cells or animal experiments, our results preliminarily indicate that it may affect *NUDT21* against LUAD.

In conclusion, by showing the overexpression and gene regulation network of *NUDT21* in LUAD, we expect to provide a new perspective on drug selection for clinical workers from the standpoint of immunotherapy for LUAD. Furthermore, identifying new therapeutic targets and prognostic biomarkers to more accurately predicts the survival in patients with LUAD. We also provide potential therapeutic drugs from traditional Chinese medicine in the treatment of LUAD.

Declarations

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Author Contributions

Yong-li SITU performed data analysis work and aided in writing the manuscript. Hong NIE and Zhixin FANG designed the study and assisted in writing the manuscript. Yong-li SITU, Li-na LONG, and Hai-jian LI edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed in this study are available from publicly available platforms in the manuscript's method introduction.

Consent to publication

None.

Ethics approval and consent to participate

In this study, relevant data were obtained from some publicly available platforms for retrospective analysis. All data in the platform had obtained informed consent signed by the patients or their families and published by relevant institutions or individuals.

Competing interests

The authors state that there are no conflicts of interest to disclose.

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Figures

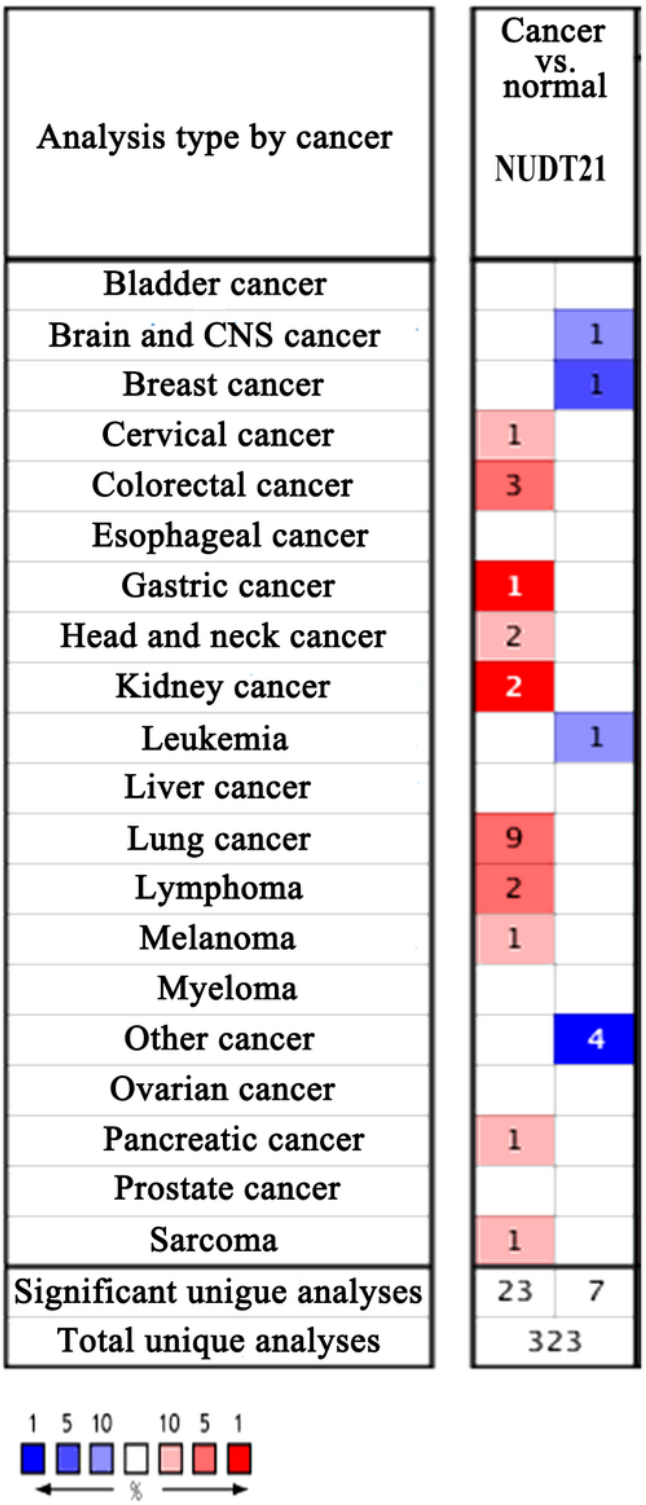

Figure 1

mRNA levels of NUDT21 in lung cancer (ONCOMINE). The figure shows the numbers of datasets with statistically significant mRNA over-expression (red) or downregulated expression (blue) of NUDT21.

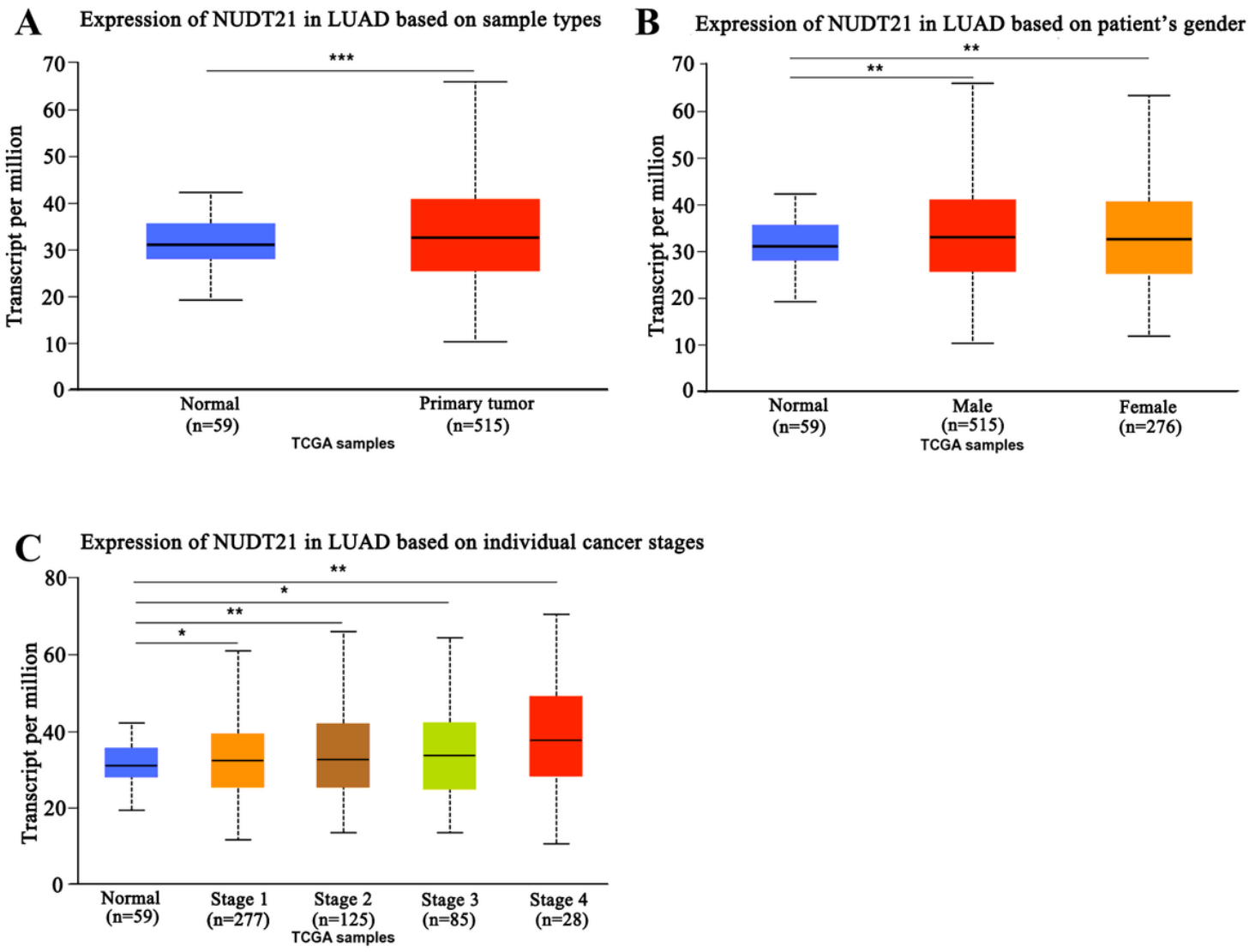

Figure 2

The transcription levels of NUDT21 in LUAD (UALCAN). A. Boxplot showing relative expression of NUDT21 in normal and LUAD samples. B. Boxplot showing relative expression of LUAD of either gender in normal individuals and LUAD patients. C. Boxplot showing relative expression of NUDT21 of stages in normal individuals and LUAD patients. Data are mean ± SE. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figure 3

The relative level of NUDT21 in LUAD (GEPIA).

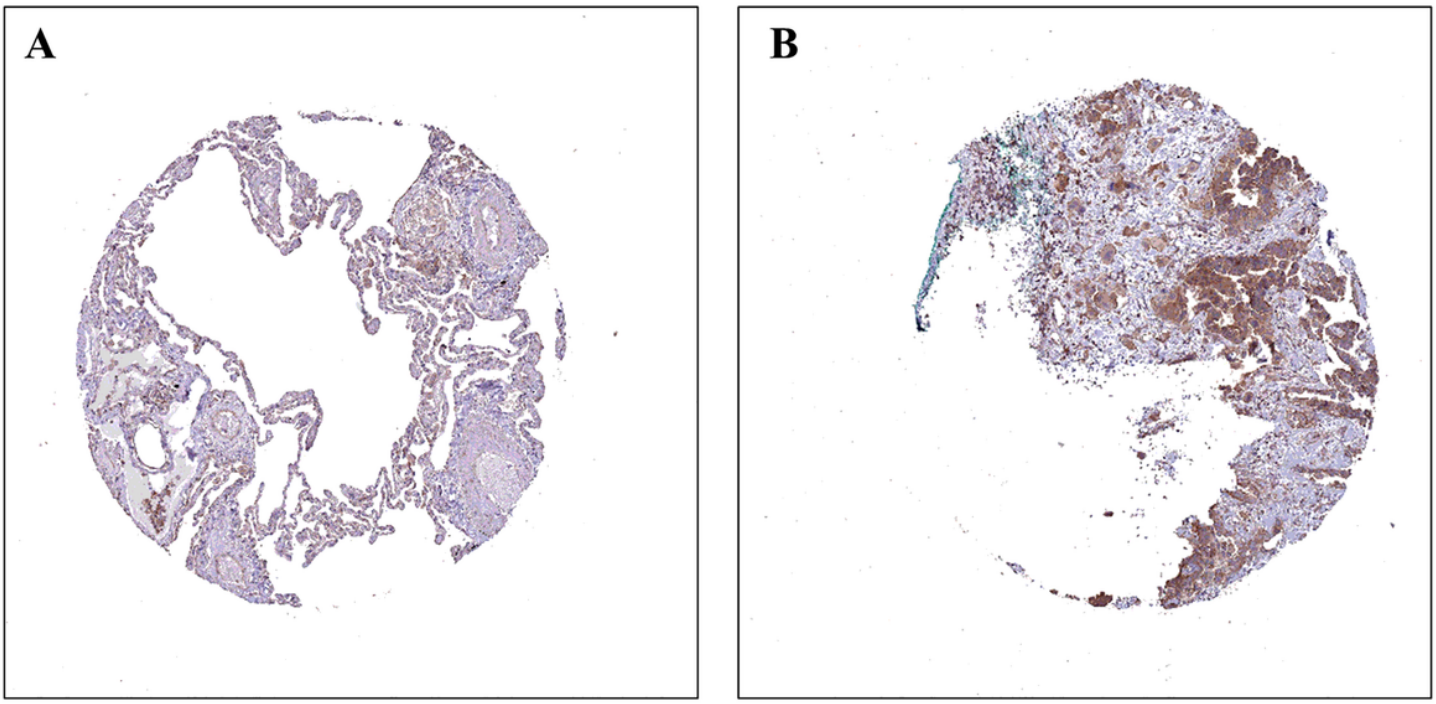

Figure 4

The protein expression of NUDT21 in LUAD tissue (The Human Protein Atlas). The protein expression of NUDT21 in (A) normal lung tissue, (B) LUAD tissue.

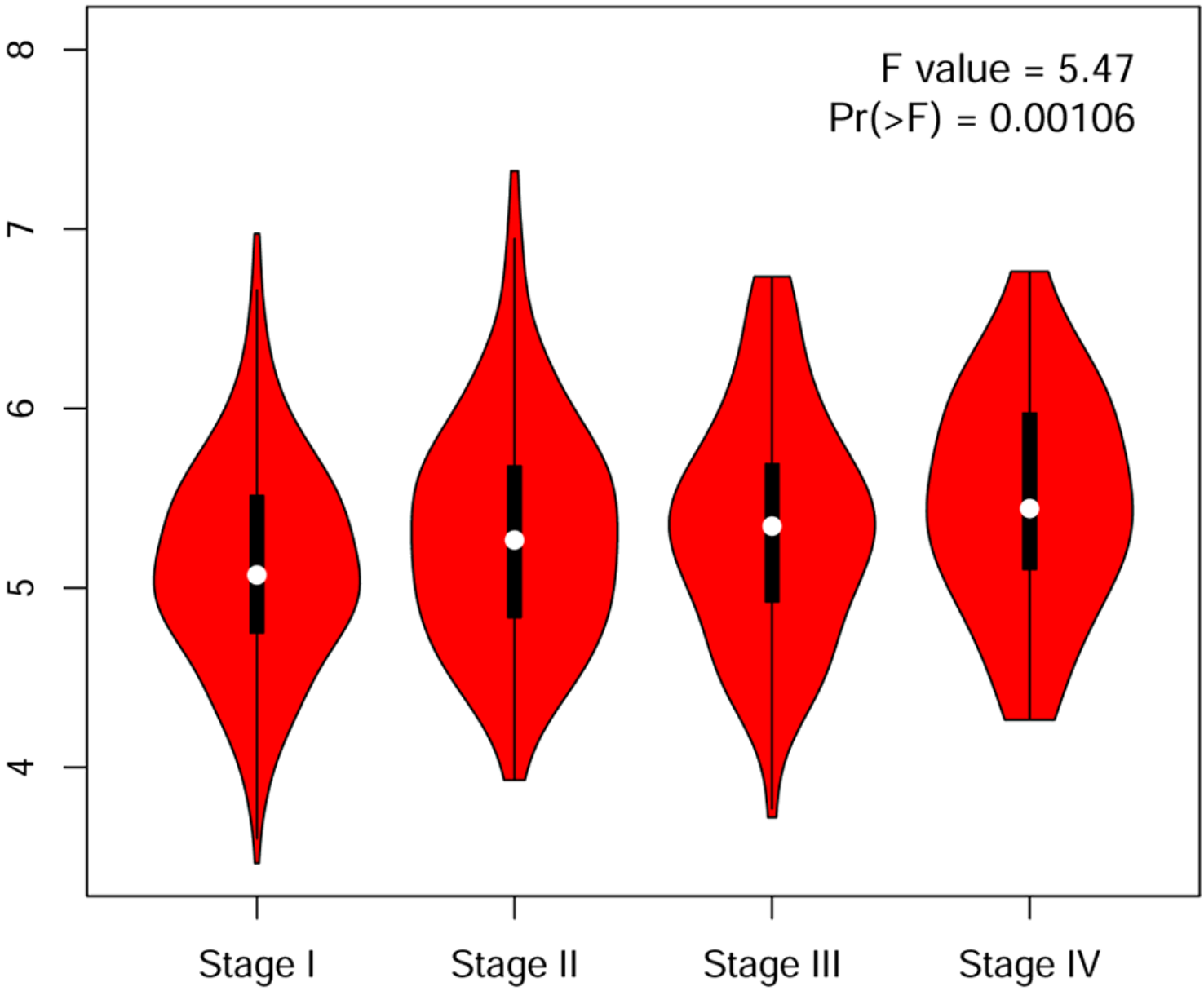

Figure 5

Correlation between the expression level of NUDT21 and the pathological stage in LUAD (GEPIA). *P < 0.05.

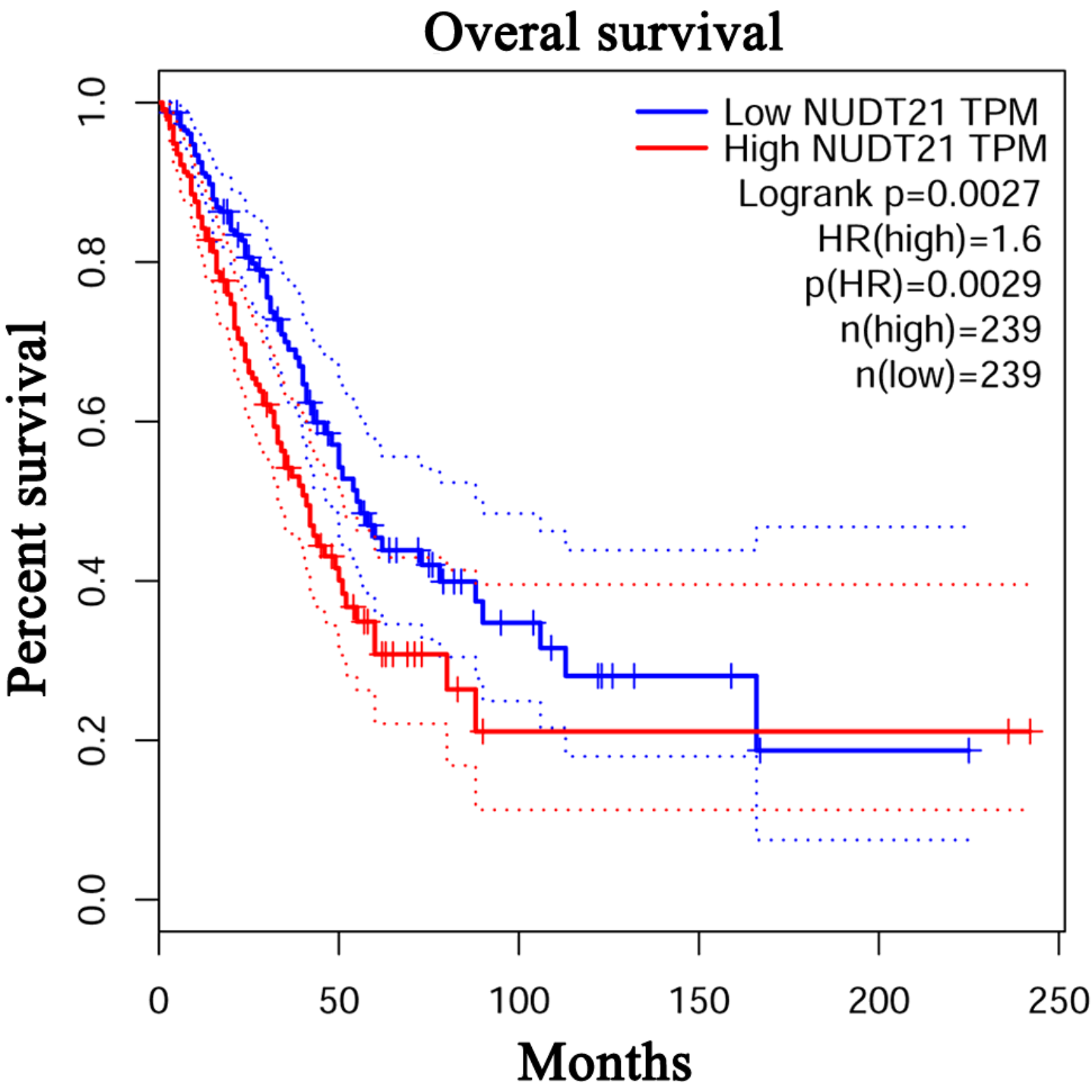

Figure 6

The prognostic value of NUDT21 in LUAD patients in the overall survival curve (GEPIA).

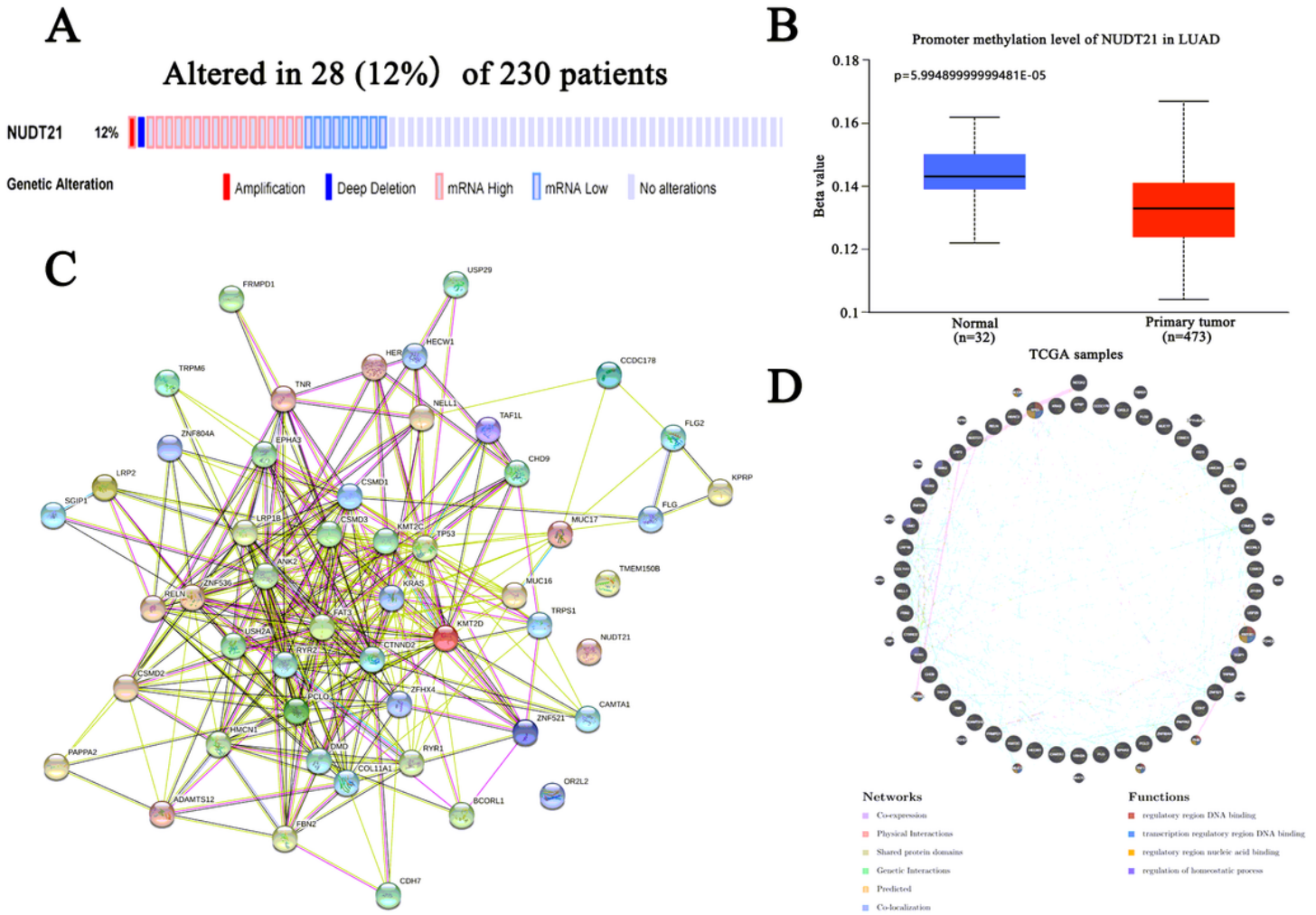

Figure 7

Genetic alteration, and interaction analyses of NUDT21 in LUAD. (A) Summary of alterations in NUDT21 in LUAD. (B) Protein–protein interaction network of NUDT21 and the top 50 neighbor altered gene. (C)Function analyses of NUDT21 and the top 50 neighbor altered gene

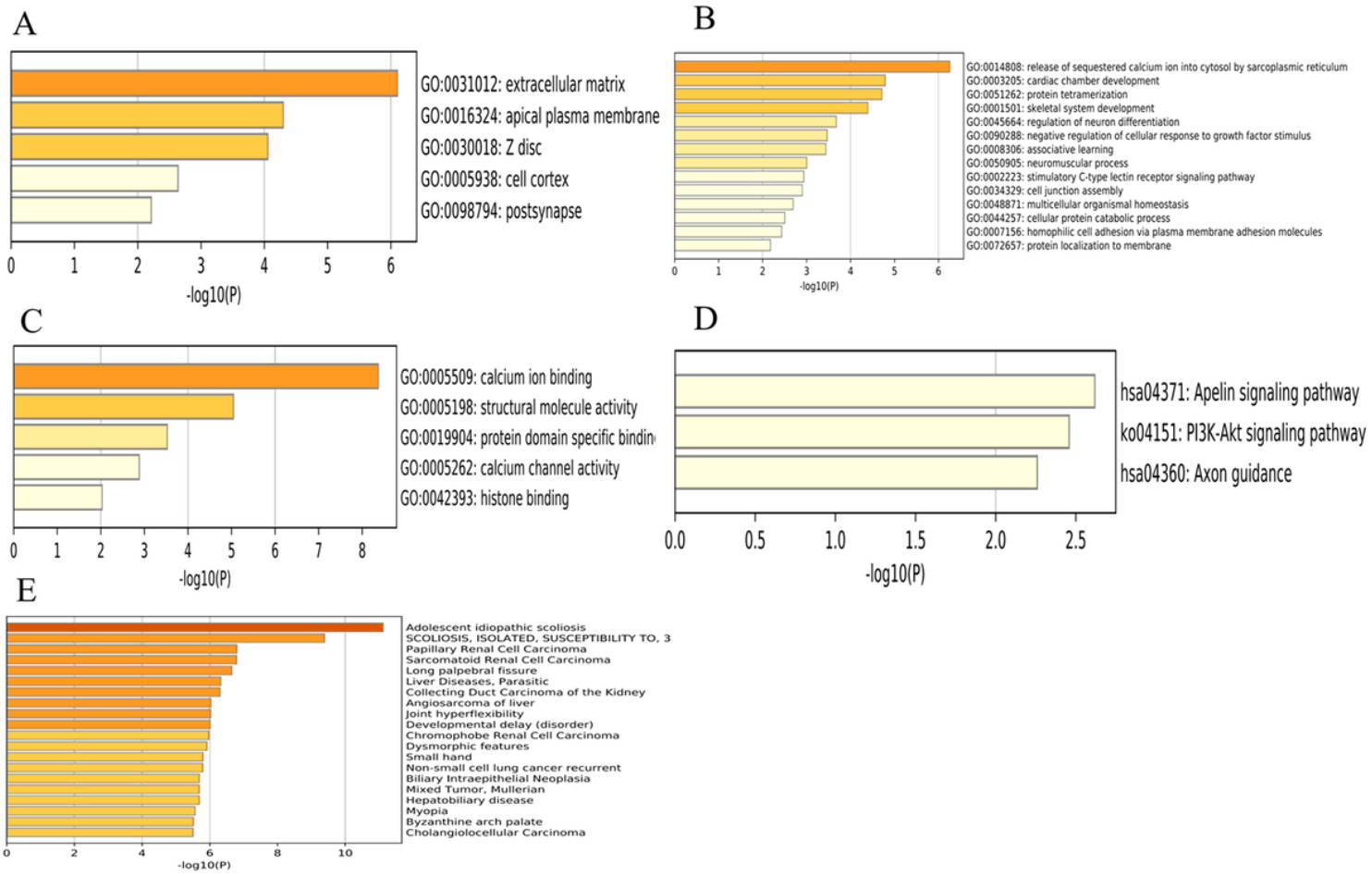

Figure 8

GO function and KEGG pathways enrichment analysis of NUDT21 and the top 50 neighbor altered gene in LUAD (metascape). (A) Cellular components. (B) Biological processes. (C) Molecular functions. (D) KEGG pathway analysis. (E) Disease.

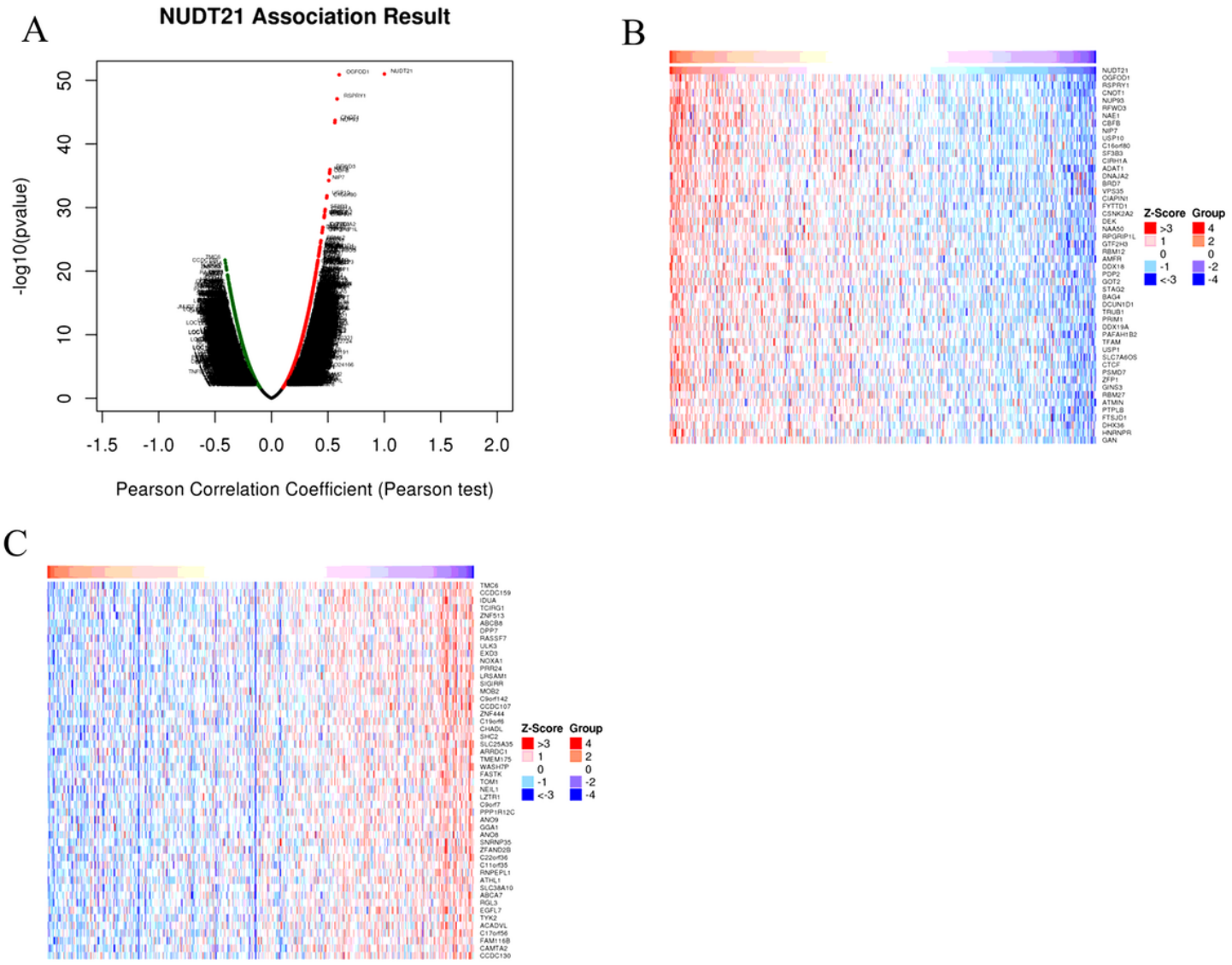

Figure 9

Genes differentially expressed in correlation with NUDT21 in LUAD (LinkedOmics). (A) A Pearson test was used to analyze correlations between NUDT21 and genes differentially expressed in LUAD. (B and C) Heat maps showing genes positively and negatively correlated with NUDT21 in LUAD (TOP 50). Red indicates positively correlated genes and blue indicates negatively correlated genes.

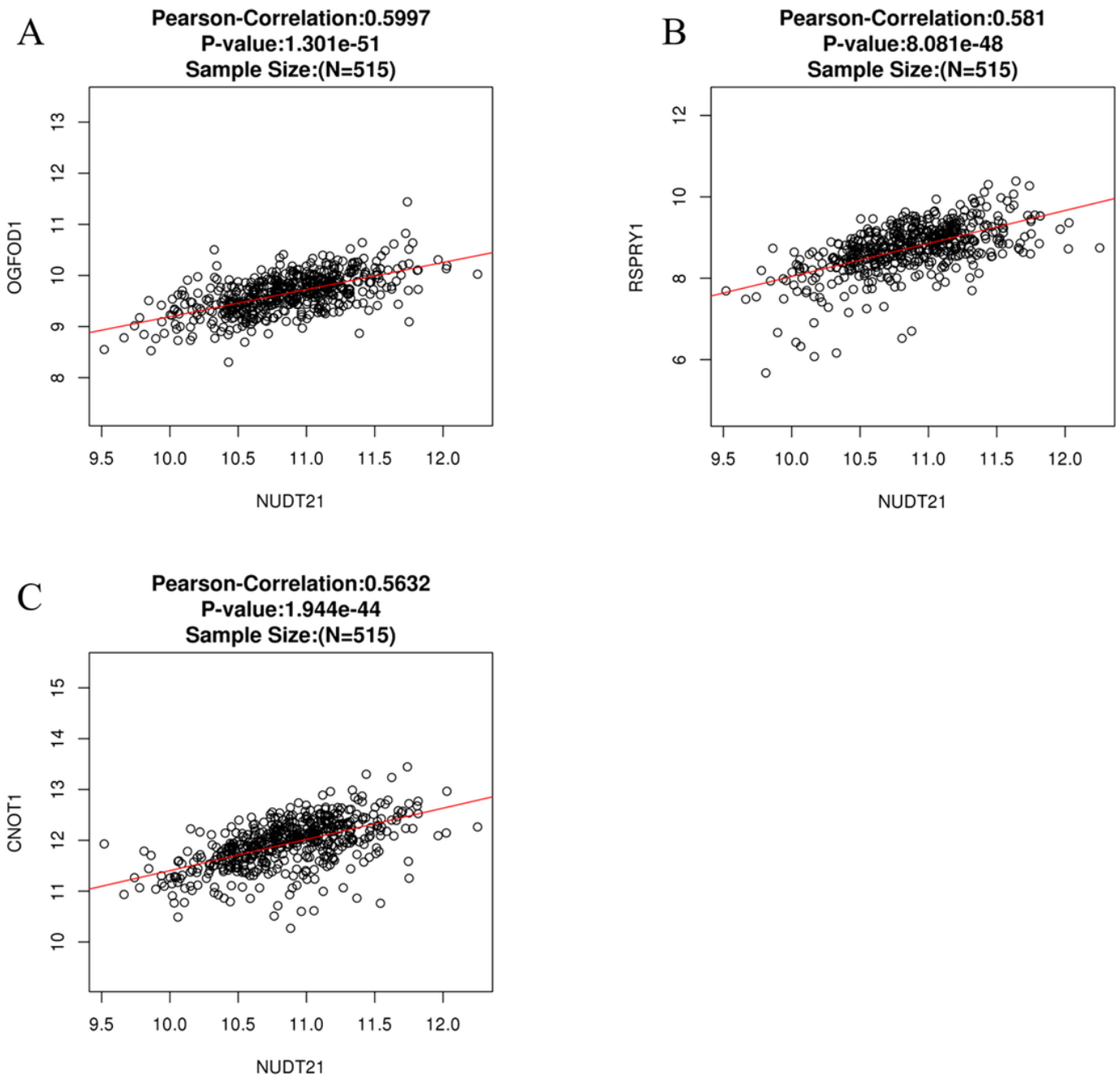

Figure 10

Gene expression correlation analysis of NUDT21 in LUAD (LinkedOmics). The scatter plot shows Pearson correlation of NUDT21 expression with expression of OGFOD1 (A), RSPRY1 (B), CNOT1 (C) in LUAD.

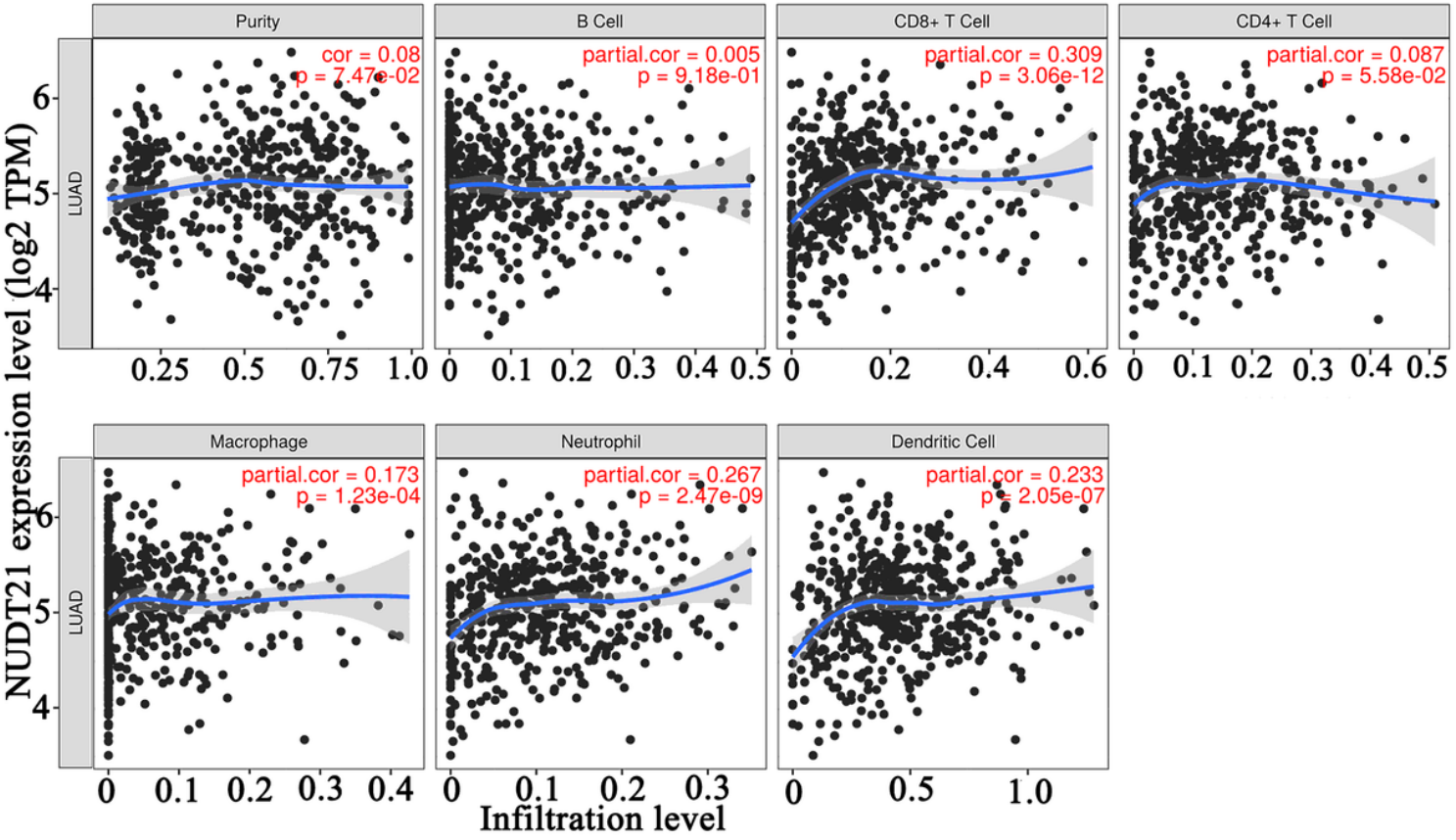

Figure 11

The correlation between NUDT21 and immune cell infiltration in LUAD (TIMER).

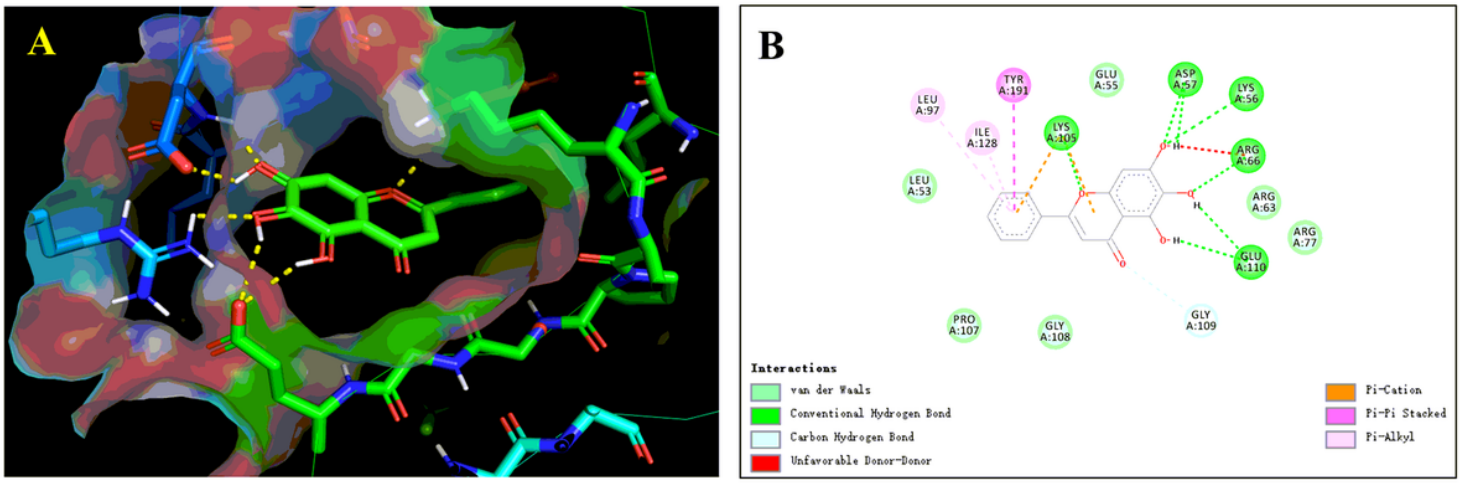

Figure 12

Molecular docking models of NUDT21 with baicalein. A. Molecular docking of NUDT21 with baicalein; B. 2D diagram of receptor-ligand interactions (NUDT21 and baicalein).